New Behavioral Insights Into Home Range Orientation of the House Mouse (*Mus musculus*)

By

Blythe E. Alexander

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Rudolf Jander

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Helen M. Alexander

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Kenneth B. Armitage

_____

Linda Trueb

_____

Stephen Egbert

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The Dissertation Committee for Blythe Alexander

certifies that this is the approved version of the following dissertation:

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Rudolf Jander

Date approved:

Abstract

Home-range orientation is a necessity for an animal that maintains an area of daily activity. The ability to navigate efficiently among goals not perceived at the starting point requires the animal to rely on place recognition and vector knowledge. These two components of navigation allow the animal to dynamically update its current position and link that position with the locomotor distance and direction needed to reach a goal. In order to use place knowledge and vector knowledge the animal must learn and remember relevant spatial information obtained from the environment and from internal feedback. The research in this dissertation focuses on behavioral components of topographic orientation, using the house mouse as a model species. Specifically, this research made important discoveries in three main areas: 1) locomotor exploration behavior, 2) the use of learned spatial information for compass orientation, and 3) testable hypotheses based on the controversial cognitive map.

In Chapter 1, I used a radial arm maze to find a systematic locomotor component to exploration behavior, which is typically described as random movement. Exploration refers to the learning process that occurs as an animal acquires relevant spatial information for home-range orientation. I predicted that this process must have a systematic component; and the results revealed that in a radial arm maze, mice avoided exploring a place explored one and two visits prior. Therefore, locomotor exploration does have a systematic component.

In Chapter 2, I trained mice to navigate to their home within a circular arena, with access to a visual beacon and an enriched visual background. The mice showed that to navigate home, they preferred to rely on the extra-arena (background) cues for compass direction. However, when these extra-arena cues became unreliable, the mice showed flexibility in their preference by ignoring the visual background and instead relying on the visual beacon to locate home. This flexibility in cue use negates a popular theory, called the snapshot theory, which does not allow for such flexibility in navigation.

To further study the use of compass cues in mice, in Chapter 3, I utilized a plus-maze to manipulate both allothetic (environmental) and idiothetic (internal) cues. The purpose was to determine which cue type predominated the directional choice of mice at the maze intersection while both leaving and returning home. Previous studies have ignored the potential difference in cue use during the complete roundtrip an animal would make within its home range. The results show that mice relied on different cues for the outward path and the homing path of a familiar complex roundtrip.

Finally, I developed two testable hypotheses and a valid experimental design that can be used to test house mice, and other animals, for the so-called cognitive map. An animal that has a cognitive map would be able to compute a novel shortcut to a goal relying exclusively on the flexibility of such a map, and not from the other two options of novel shortcutting: guidance orientation or path integration. Thus by designing my experiments to eliminate the potential for the mice to rely on a guiding cue to direct them home, and by eliminating the ability to compute a shortcut by summing the vectors previously walked, I was able to test mice for a

truly novel, map-based shortcut home. These two hypotheses were named viewpoint extrapolation and viewpoint interpolation and require pure visual exploration to acquire the necessary place and vector knowledge. Both experiments showed that mice were not capable of using pure visual exploration and therefore these studies provide no evidence that mice have a cognitive map.

Overall, my research provides evidence that mice do have a mental route-based map and to build such a mental map, locomotor exploration is necessary and sufficient for acquiring relevant spatial knowledge to later use to efficiently navigate.

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GENERAL INTRODUCTION

Home range (or topographic) orientation for mammals is a necessity for all those species, including humans, that maintain an area of daily activity called a home range. Home range orientation allows an animal to efficiently navigate among goals within that home range. Navigation is the learned movement toward a goal not perceived at the starting point. When an animal is moving within its home range, it is typically not moving around randomly—unless exploring or searching—but with directed movements toward a goal, e.g. a home base, or food source. This display of successful navigation provides evidence that these animals are storing spatial knowledge in their memory as some sort of *mental place map*. The term mental place map does not imply the concept of a cognitive map, as first proposed by Tolman (1948), which requires an animal can compute novel routes among learned places, but rather that acquired knowledge about visited places and their relationship to each other has been mentally stored.

Navigation with a mental place map requires three faculties: the ability to self-locate, vector knowledge, and the *a priori* mental ability to store this information and later retrieve it for use. An essential first step of navigation is the animal's ability to self-locate, which is assessing its current position relative to the layout of the environment. The second essential step is to link the current position with the stored vector knowledge—the distance and direction—connected to the goal. The *a priori* mental abilities are the pre-existing neural circuitry available for organizing, storing and using the acquired relevant knowledge. When entering a new environment, an animal lacks the information needed to navigate and thus

nothing is stored in memory. Therefore, a prerequisite for building a mental place map of that environment is *spatial experience*.

Spatial experience

Spatial experience in a new environment is obtained through exploratory behavior, a learning process. Exploring is distinct from searching. Whereas an animal engaged in the former acquires topographic knowledge, in the latter mode it seeks to discover the locations of needed resources.

Home range exploration can be performed in two distinct procedures: pure visual exploration and locomotor exploration, which also may be combined. In *pure visual exploration*, the mental mapping of the surrounding topography takes place by visual scanning from one or more vantage points. A prerequisite for mapping with this method is to have well-developed, three-dimensional visual perception. There is only evidence for humans that this method is available. *Locomotor exploration* is the process by which sensory-motor activity incrementally maps properties of a future home range into long-term memory to be used by the animal subsequently for efficient navigation within the explored range (Alyan, 2004b, Wallace et al., 2008).

When ignorant about a place, an animal first must explore. There are three options for moving around a new environment. The first is that the animal is "lost" and is moving about without direction, during which it is unlikely that information acquired is usable without further organization. The two means of learning spatial knowledge through locomotor exploration can occur during guided orientation, if there are guiding stimuli present or vector orientation, if not (Alyan and Jander,

1994, Wallace et al., 2008). An animal using guided orientation simply uses a beacon, or follows an odor trail, road or wall to navigate toward a goal. During vector orientation, the animal computes the direction and distance it has walked from an anchored starting position to navigate back to that starting place. These two methods of orientation are not mutually exclusive, and more likely occur together.

Guided orientation

With guided orientation, vector information can be directly assessed if the goal is perceived by the animal in terms of direction and distance and thus it uses environmental cues to direct movement toward the goal (Alyan and Jander, 1994). Otherwise, environmental or allothetic cues include visual, auditory, tactile or olfactory information provided by cues external to an animal. Of these types of environmental cues, I only study visual cues.

Environmental visual cues can be classified as distal or proximal. Distal cues are features of the environment that, because of their distance, perceptively change minimally while moving. These cues typically are unapproachable by the animal. Distant mountain ranges or large buildings on the skyline are examples of distal cues, and some celestial cues, such as the sun or moon. Proximal cues are close enough to an animal to be approachable. When they are near or at a goal they can serve as beacons to simply walk toward, a format that is guided orientation.

Direct, beacon-guided locomotion toward a goal can provide experience of a simple vector. Storing this vector information in memory would allow the animal to navigate still to this same goal, even if for some reason the beacon or guiding cue

were removed. The experience obtained from this beacon-guided orientation would also allow the animal to remember and use for self-location the spatial constellation of various non-beacon visual cues, often referred to as "landmarks".

Vector orientation

Another way to gain locomotor experience is during vector orientation. The learning process is called path integration, during which the dynamic position is updated by the integration of linear and angular self-motion information linked to an anchored starting position (Jander, 1957, Etienne et al., 1996, Etienne and Jeffery, 2004). During path integration, environmental cues help the animal to update the vector computation to correct for errors; however in rodents, the information used for path integration is from internal or idiothetic cues, or sensory feedback caused by an animal's own movement. This movement provides vestibular and kinesthetic feedback information. For example, as an animal walks (kinesthetic information) and turns (vestibular information) to keep track of vectors traveled, the animal can use this information to determine the vector(s) needed to navigate back to that anchored goal from the current position, a process termed dead reckoning. An animal self-locates via path integration by assessing and computing any vectors traveled with relation to that anchored position. The simplest example of this type of navigation is that an animal walks a straight trajectory away from the starting point and in order to return, the animal must reverse the path.

Spatial updating errors always accumulate through time and space with path integrating (Singer et al., 2006), which could result in fatal mistakes for an animal running from a predator to safe cover. Also, path integration is anchored to the

animal's starting position and if the goal suddenly changes (from a home base to a food source) a new vector cannot be computed. Therefore, using map-based orientation is a more effective method once enough information is acquired because of the addition of using place knowledge with vector knowledge for orientation (O'Keefe and Nadel, 1978). By associating vector knowledge and place knowledge, integration errors are corrected and multiple places can become the anchored positions to link with vector knowledge so that the animal is not limited to only knowing how to navigate to the starting position.

Map-based orientation

Path integration and guided orientation allow an animal to maneuver through a new environment without necessarily becoming lost, so that useful information about multiple places and their spatial relationships can be learned. Only short-term memory is required for guided orientation and path integration, but storing the needed spatial knowledge about multiple areas in long-term memory would be beneficial for survival (O'Keefe and Nadel, 1978, Etienne and Jeffery, 2004). Thus, the need to build a mental place map for map-based orientation.

With map-based orientation, self-locating via *place recognition* relies on environmental cues, such as landmarks, to recognize places (Alyan and Jander, 1994, Singer et al., 2006). Within an explored environment, learned places can become anchored places linked among each other with learned vector information. And an animal moving throughout this environment can dynamically update its current position and calculate the needed path to whichever anchored place is the goal. Successfully navigating among places in this explored environment implies

that cues are associated with this knowledge to recognize places and that this knowledge is stored and organized to represent spatial relationships.

Rodent navigation studies—making use of the house mouse

Attempts to find common mechanisms for spatial orientation and memory among mammals has been supported by a comparison of human and non-human primates to rodent studies. Most papers comparing primate and rodent cognitive topographical maps suggest that the two groups are able to spatially orient similarly (Wang and Spelke, 2002, Lavenex and Lavenex, 2009). Mammals, of course, share traits within this group of animals; however, it is important not to assume all these traits are similar or that these traits function the same way among species within the mammalian group. For example, primates have a well-developed, three-dimensional visual perception, while rodents do not; therefore, with rodents pure visual exploration may not be sufficient for acquiring necessary spatial information.

In mammals, there are numerous publications focusing on rodent navigation, but most of these studies use the rat (*Rattus norvegicus*) as the subject animal. And despite the large amount of scientific research on rodent navigation there are still many open questions. The research of this dissertation addresses some of these questions, which will be elaborated on in later sections discussing each chapter topic.

To build on the growing understanding of rodent home range orientation, this research will use the house mouse as the subject animal. Another member of the Muridae family, house mice have been represented in navigation studies some hundred-times less than rats. Therefore, studying the house mouse provides the

opportunity to compare mouse behavior to what is known about the rat. Also, mice are smaller than rats so they require less room and thus are less expensive to house and use; and more importantly in navigation studies, the same size arena becomes larger for the mice, creating a better space to test navigation.

For most of the experiments, I will employ a less used but effective methodology by taking advantage of the mouse's instinctual maternal motivation to retrieve its pups to a nest box; most studies use other motivational applications, such as food deprivation or fear of drowning. My methodology requires less time for training than either of the other two options, and imposes less stress to the animal. Chapter by chapter, I seek answers to the following questions discussed in the following sections.

Chapter 1: Locomotor patterns in the exploring house mouse (Mus musculus)

In rodents, past research on home range orientation almost exclusively covered their navigational performance with pre-existing knowledge, as most research began well after exploration had taken place. Compared to this immense body of post-exploration research, our understanding about rodent exploration, proper, is minimal.

Spatial exploration behavior appears random; however, it is hard to imagine how purely random locomotor exploration could be helpful in acquiring systematic map knowledge that, of necessity, is highly structured (non-random). To shed light on this problem, I used a six-arm radial maze to discover any systematic patterns in rodent locomotor exploration that may occur over space and time.

Chapter 2: Flexible Preference Between Distal Visual Cues or a Salient, Visual Beacon as Directional Cues for Homing in the House Mouse (Mus musculus)

Previous studies have shown that a navigating mouse prefers to use distal (extra-arena) visual cues for compass orientation (Alyan and Jander, 1994). If the mice learn these cues are reliable predictors toward a goal, can they learn to ignore them if they become unreliable? A persistent theory in spatial cognition, the snapshot theory, predicts the mice would not be capable of such a flexible behavior. Snapshot theory states that the navigating animal uses a form of image matching to compare remembered 'snapshots' or indiscriminately stored retinal images, taken when leaving the home base. Thus, to navigate home, self-location involves comparing the perceived current retinal input to the stored images and then the animal returns home by moving until the images visually match (Collett and Cartwright, 1983, Collett and Collett, 2002, Wang and Spelke, 2002, Cheung et al., 2008, Stürzl et al., 2008). This theory persists despite no evidence to support it. Therefore, this study tests the theory by training mice to navigate to a nest box within an open arena, reconfirming their preference for distal compass cues, and then these cues are made unreliable to test the heading choices of the mice.

Chapter 3: Feedback control of a navigating house mouse away from home and back: idiothetic or allothetic?

In this chapter, the problem to be solved is which type of cue, allothetic or idiothetic, influences the mouse's directional heading during the two segments of a round trip, the outward path and the homing path. Previous studies of the use of compass cues tended to focus on only one segment of the round trip, and typically

ignore the outward path. The mice are trained in a plus-maze to navigate between a goal and a nest box located at the end of two different arms of the maze. The most salient cue available will be a directional light source. Experimental manipulations while the mouse is navigating between home and the goal location show the importance of certain cues on the mice's decision making. These manipulations include changing the directional light source and/or rotating the arena to affect idiothetic and allothetic information while the animal is shuttling between home and an outside goal location.

*Chapter 4 The Shortcut and the Map in the Rodent Navigation System: Experiments with the House Mouse (*Mus musculus*)*

A highly debatable subject with mental map-based navigation is with the limitations of this navigation system. Tolman's proposed "cognitive map" implies that an animal can compute a novel shortcut to any known goal. Various authors have attempted to test for this ability of novel shortcutting, but all have failed in their design at showing a truly novel route (Sutherland et al., 1987, Alyan, 1994, Biegler and Morris, 1996, Akers et al., 2007, Hamilton et al., 2008).

The purpose of this study is to propose and test a hypothesis of pure map-based shortcutting: viewpoint extrapolation. An animal navigating to a known goal by viewpoint extrapolation would use spatial information acquired solely from visual access to terrestrial cues from one viewpoint, and then when placed at a previously unknown location that shares the same visual cues, can compute a shortcut home. To test this hypothesis, I trained mice to navigate to a goal in an

open arena. I then shifted the arena by its diameter leaving no overlap in space to test their ability at the novel location to find the direct shortcut back to the goal.

Chapter 5: Can house mice (Mus musculus) compute a novel shortcut with two reference viewpoints?

This chapter continues testing for mice's ability to compute a novel shortcut home. However, the mice are given the opportunity to acquire visual spatial information from *two* viewpoints before being tested in a previously unexperienced, intermediate location. This new proposed method is called viewpoint interpolation. Mice are trained in an open arena that is shifted between two training positions within a room. The mice learn to navigate to two different goal locations relative to the arena training positions. Then the mice's choices in directional heading are tested when the arena is shifted to a location directly between the two training positions, with no overlap in space.

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CHAPTER 1: Locomotor patterns in the exploring house mouse (*Mus musculus*)

Abstract

For animals, home range exploration is a learning process during which the animal uses sensory-motor activity to incrementally map properties of a future home range into long-term memory. The animal then uses this map for efficient navigation within the explored range. Most research on home range orientation focuses on the navigational performance of the animal with pre-existing knowledge—after exploration. Typically, exploration behavior appears random, but there must be some systematic component to result in the animal acquiring spatial knowledge. Few studies have looked at the spatiotemporal pattern of exploration for any part that is not random. Using the house mouse as the model species, I study exploration behavior in a radial arm maze, with the nest box in the center. With these spatial constraints, it is easier to separate the random and systematic components of exploration. I found that the mice tend to avoid exploring an arm of the maze previously visited one or two times earlier, yet the choices among any other arms not previously visited are almost indistinguishable from random.

Introduction

Home range exploration is an active learning process during which an animal uses sensory-motor activity to incrementally map properties of a future home range into long-term memory. These properties can then be used for efficient navigation in a future home range, which is necessary for survival. An animal's ability to successfully navigate without getting lost after spatial exploration is evidence this mapping occurs (Poucet et al., 1986, Whishaw and Brooks, 1999).

Most studies on topographic (or home range orientation) investigate post-exploration behavior. However, the exploration process itself is not well understood and aspects of it appear random. Open arena studies on the spatiotemporal exploratory behavior in rodents show characteristics under lit conditions such as perimeter patrolling (Drai et al., 2001, Zadicario et al., 2005, Avni et al., 2006, Clark et al., 2006, Avni and Eilam, 2008) and home base behavior, (Drai et al., 2001, Zadicario et al., 2005, Avni et al., 2006, Clark et al., 2006, Nemati and Wishaw, 2007, Avni and Eilam, 2008). Under dark conditions, rodents have exhibited a 'looping' behavior that precedes those behaviors exhibited in the light (Zadicario et al., 2005, Avni et al., 2006). The establishment of an area as a home base indicates the importance of having this anchored starting place as well as a place to retreat (Whishaw et al., 2006). With a home base in the arena, a developmental sequence of behaviors in exploration emerges that consists of increasing distance and time with each roundtrip from the shelter (Fonio et al., 2009).

While these studies describe the behavioral movements, they do little to elucidate on how exploratory paths in the arena are chosen. An initial attempt to separate systematic patterns of movement from seemingly random movements proved difficult when using house mice in an open arena with a home base. In particular without high-level technology, recording the exact location of the mice over time was difficult to quantify (Alexander, unpublished data).

To shed light on this problem, I use the house mouse as the model species to study exploratory behavior in a radial arm maze, with the nest box in the center. With these spatial constraints, it will be easier to separate the random and systematic components of exploration. Therefore, the main question asked is there a pattern of arm choice by exploring mice in a radial arm maze? The null hypothesis was the choice of arms to explore is indistinguishable from random.

Methods

Animals

Eight experimentally naïve, captive-reared, brown to black female house mice of a mixed strain were used. Each of these female mice had a litter of pups, which were useful for establishing the nest box as home. All mice were raised in transparent cages (48 l × 25 w × 15 h cm). Pine shavings and cotton were used as nesting materials, and food and water were given ad lib. The average age of the mother mice was 8 mo., and all litters of pups were no older than 2 weeks of age. All mice were kept under a 12:12 h light:dark cycle and were tested during the light phase. These mice all passed vision tests before being used.

Arena

An elevated radial arm maze constructed of wood was used (fig 1). The nest box was placed on a central platform (17.5 cm diameter). Radiating from the platform were six identical, evenly spaced arms (60 cm long, 8 cm wide). The nest box consisted of a wooden platform (16.5 cm diameter) and a removable plastic lid (13 cm diameter) with a circular entrance (5 cm diameter) cut into the center of the top. The top surface of the nest box was raised 6 cm from the surface of the maze arms. The elevated radial maze was located within an open arena (2.25 m^2) with perimeter walls, 30.5 cm tall and a sand covered floor, which was used only to contain mice if they jumped off the radial maze. Three black cylinders (21.5 cm height; 17 cm diameter) were placed under alternating arms of the maze for support; these were visible to the mice.

Outside the arena

The room was fully illuminated by evenly spaced fluorescent ceiling lights and had furnishings that enriched the visual background external to the open arena. A video camera (on a black tripod 1.7 m tall), was placed 1.25 m away from the distal end of the maze arm designated 180 degrees. The observer was 1.25 m away from the end of the 240° arm.

Procedure

A small amount of nesting material and two pups were placed in the nest box so that the mouse readily accepted it as her home base. The mouse was then put into the nest box, which started the observation period. This session was videotaped for later analysis. The mouse was given one hour to freely explore the

arena with whatever level of activity she exhibited. If the mouse did not leave the nest box for 30 consecutive minutes, the session ended.

Data collection and analysis

The order the mice visited each arm was manually collected from video. An arm was considered explored only if the mouse walked at least halfway to the end. To determine if there were a clockwise/counterclockwise (CW/CCW) pattern of movement around the maze, *each pair* of arm visits were analyzed relative to the previous. This analysis was irrespective of whether the mouse walked back into the nest between arms or circled the nest from the other direction. Thus, this CW/CCW analysis only examined choices taken with relation to the preceding angle. If the mouse walked down the same arm or the arm 180° from the preceding arm, then it was not included in the analysis. To determine if any directional choice was influenced over a longer period, each angle was also analyzed relative to the arm angle 2 visits prior. For example, if a mouse visited the arms in this order: 180°, 60°, 120°, then the *2nd visit* analysis would be between 180° and 120°, which is CCW. A chi-square test was used to determine if the CW/CCW analyses were distinguishable from chance.

To determine whether there is a pattern in the order of arms visited, the sequence was analyzed by ranking the arms, 0–3, by the distance relative to the arm previously visited. If the arm visited was the same arm as the previous visit, it was ranked as '0.' Visiting an arm 60°, or one arm away from the preceding visit was '1', while '2' indicated a 120° or two arm distance. Directly opposite to the preceding

visit was ranked '3.' This sequence was further analyzed for every *2nd visit*, and *3rd visit*.

To help explain these analyses of sequence, figure 2 gives an example of four arms visited in a series, a–d. The numbers for each arm are the rankings relative to the first visit, a. The analysis of each angle to its preceding angle, or *each pair*, would give the following rankings for a–d: 3,1,3. The next analysis of every *2nd visit*, would rank c as 2 relative to a, and d as 2 relative to b. Finally, the *3rd visit* analysis can only rank d relative to a, which is 1.

Under the assumption that all mice exhibited the same exploration patterns, the data were lumped together. To test that the movement pattern of mice with longer activity within the hour does not change over time, a chi-square test was used to compare the first 10 ranked values for *each pair* to the last 10 ranked values for the four mice with the highest level of activity within the hour of testing. Once this was confirmed, a binomial distribution test was used to determine if each rank level within all 3 analyses was distinguishable from chance. The level of significance was set for a p-value of 0.05.

Results

Behavior

During the one-hour exploration time, the activity levels varied from 10–30 min. among the eight mice. Typically, before venturing out onto an arm, a mouse would leave the nest box and circle it before retreating back inside. Outings from the nest box varied. Sometimes a mouse would leave the nest box, walk down an arm, and return to the nest, and sometimes a mouse would leave the nest and walk

multiple arms before returning. Also, the mice varied behavior during multiple arm outings by either walking across the nest box to another arm, or circling the nest to the next arm. As mentioned, these variations were ignored in the data analysis; only the series of arm angles chosen was analyzed.

CW/CCW

There was a trend toward CW (fig. 3; Table 1) for *each pair*, but not significant ($p = 0.19$). A closer look at each mouse separately showed that 2 mice chose more CW directional turns, while the remaining 6 mice were close to a 50% split between the two choices. For the *2nd visit* (fig. 3; Table 1) the frequency of CW versus CCW was not distinguishable from random ($p = 0.80$)

Table 1: The total number grouped into CW or CCW for the analysis of each pair of angles and for each angle relative to the 2 visits prior

	Each Pair	2ndVisit
CW	91	72
CCW	74	75

Sequence analyses

To address the question if the pattern of movement changed over time, the chi-square test was used to compare the first and last 10 rank values for the four most active mice giving a $p = 0.99$. This result indicates that the mice use the same exploration pattern over time and so can be grouped together for the sequence analyses of each rank value, under the assumption that all mice behave similarly.

For *each pair*, the number of times that the mice walked down the same arm (0 ranking) (Table 2) differs significantly from chance ($p = 9.94 \times 10^{-6}$) (fig. 4a). The 1 and 2 rank totals differ from chance levels and the 2 rank was significant ($p = 0.09$

for 1; 0.04 for 2). The ranking of 3 was not distinguishable from chance. For the *2nd visit* analysis (fig. 4b), only the 0 rank value differed significantly from chance (p = 0.25) (Table 2). By the *3rd visit*, there was also no difference from chance (fig. 4c).

Table 2. Total number of angles within each rank for each analysis: each pair, 2nd visit, 3rd visit, and the numbers for each analysis if the choices occurred by chance. ** p <0.01; * p < 0.05

Rank	Each Pair	Random Each Pair	2nd visit	Random 2nd visit	3rd visit	Random 3rd visit
0	14**	36	24*	34.67	36	33.33
1	81	72	77	69.33	69	66.67
2	84*	72	70	69.33	61	66.67
3	37	36	37	34.67	34	33.33

Discussion

The results indicate that directional choice (CW/CCW) was not distinguishable from random. The direction of turning between each pair of angles shows an insignificant preference for CW direction (fig. 3). Further analysis of each mouse's directional choices indicates 2 of the 8 mice were more likely to turn CW than CCW, while the other 6 showed choices very close to 50% CW/CCW, which explains the trend. Possibly, these 2 mice have turning preferences, and further studies on turning choices in mice could clarify this anomaly. In addition, these results suggest the need for further studies to use a statistical approach that takes into account differences among individual mice.

Expanding to every other angle reveals no apparent turning pattern, as the choices were very close to 50% CW/CCW. These findings differed from studies showing rats have the ability to locate food at the end of the arms in a radial maze with no redundant arm searching (reviewed in: Olton, 1979). Those rats searched

the end of each arm adjacent to the previous one in a continuous direction. The results in this study suggest that there is no systematic pattern associated with turn directions when exploring.

The sequence analyses revealed a few interesting trends. First of all, the comparison between the first and last rank values for each pair showed no change in spatial exploratory patterns over time (within one hour). Secondly, a pattern emerged in the sequence of arms visited. The mice typically avoided an arm they visited one or two times earlier (fig. 4a,b). This pattern is consistent with spontaneous alternation behavior seen in rats searching in a T- or Y-maze, indicating that previous movement influences a rodent's subsequent choice (Dember and Fowler, 1958, Olton, 1979, Schenk and Grobety, 1992). Avoiding the arm visited two times earlier indicates memory retention longer than that of working memory. Indeed, rats have demonstrated at least a 105 min. temporal memory retention for arms visited (Hanneson et al., 2004). The arm just visited was not always avoided, which could be explained by a need to reset the input of stimuli for path integration (Schenk and Grobety, 1992), or perhaps the repetition provides opportunity for acquisition of more visual information in that space.

It is interesting that when avoiding the arm previously visited, instead of a uniform increase spread to the other 3 distance choices the mice were more likely to choose either the arm 60° or 120° away instead of 180°. Additionally, there was a slight preference for the arm 120° away over the 60° (fig. 4a). These results are corroborated by findings that rats, given a choice of two arms, are more likely to enter the arm leading to a place more distant from the one visited, up to 135°; and

that rats tend to neglect arms less than 45° from the start position (Schenk and Grobety, 1992). The use of a six arm radial maze in the present study provides directional options that are no less than 60°, and thus the pattern discovered might differ in a radial maze with more arms.

Given a choice between 135° and 180°, rats showed no preference distinguishable from chance (Schenk and Grobety, 1992), which is similar to the results in this study that there is no difference from chance and the choice to visit the arm 180° away from the arm visited one, two or three times earlier. Also, indistinguishable from chance were the visits relative to *any* arm visited three times earlier. Therefore, in addition to the discovered systematic pattern of exploration seen in the avoidance of repeating arm visits there is a random component. This random component is important to note because as mentioned when searching for food in a radial arm maze, rodents have moved in a highly systematic manner (Olton, 1979, de Saint Blanquat et al., 2010). Thus rodents are capable of behavior distinguishable from chance, suggesting a reason for the random component.

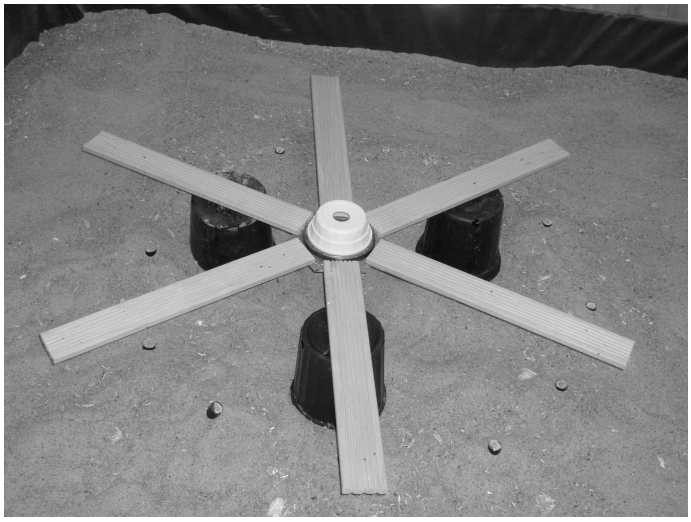
This randomness in spatial exploration may be a survival strategy. Any predictable pattern is of value to a predator, making exploration a costly learning process for an animal. Therefore, the random component could be beneficial for its unpredictability. A similarly less systematic movement pattern is described for patrolling behavior, which is observed after the animal stops searching because they receive their sought after reward (Olton, 1979, de Saint Blanquat et al., 2010). However, patrolling differs from exploration because the animal is moving around in a familiar area. Therefore the random movements observed in patrolling cannot

be explained by a lack of spatial information, suggesting a purpose for this randomness, e.g., unpredictability.

The radial arm maze is often used in rodent studies of working memory and searching behavior (Olton, 1979). Exploring is distinct from searching. Whereas in the latter mode the animal seeks to discover the locations of needed resources, the former acquires topographic (or spatial) knowledge. Therefore, studies that deprive an animal of food or water to increase motivation to move about a maze are eliciting the searching behavior, not exploration. It would be useful for more studies on spatiotemporal movement in mazes to focus on spatial exploration behavior. Any systematic and random components of spatial exploration understood under conditions with spatial constraints potentially can be extrapolated to research on spatiotemporal movements during exploration in open arenas and more natural settings.

In conclusion, the results show that the mice tend to avoid exploring an arm of the maze previously visited one or two times earlier, yet the choices among any other arms not previously visited are almost indistinguishable from random. Also, there is no apparent systematic pattern found in turning choices. Thus spatial exploration behavior in mice contains both systematic and random components when moving in an area with spatial constraints.

Figures

a. 

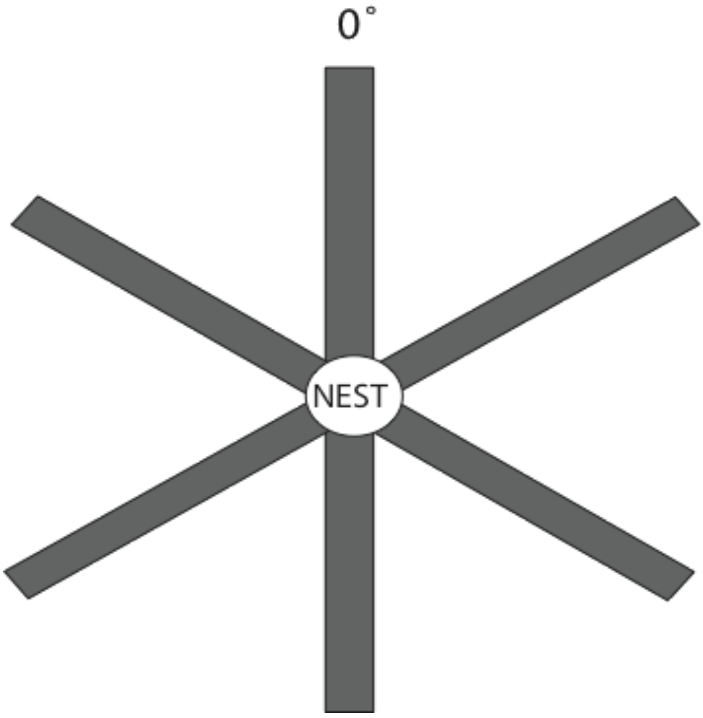
b. 

Figure 1. The elevated radial arm maze set-up.
a. Photo of actual maze with circular nest box in center.
b. Schematic to be used in analysis, showing which arm was designated 0° throughout experiment.

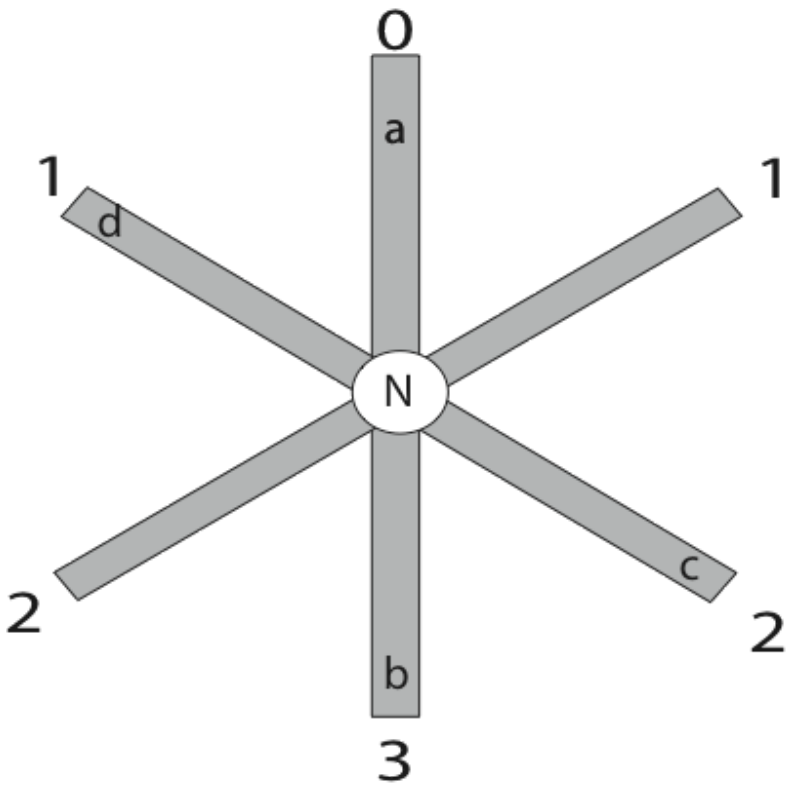


Figure 2. A schematic example sequence analyses. The letters a–d represent the arms visited in series. The numbers for each arm indicate the rank value relative to the first arm visited, 'a.'

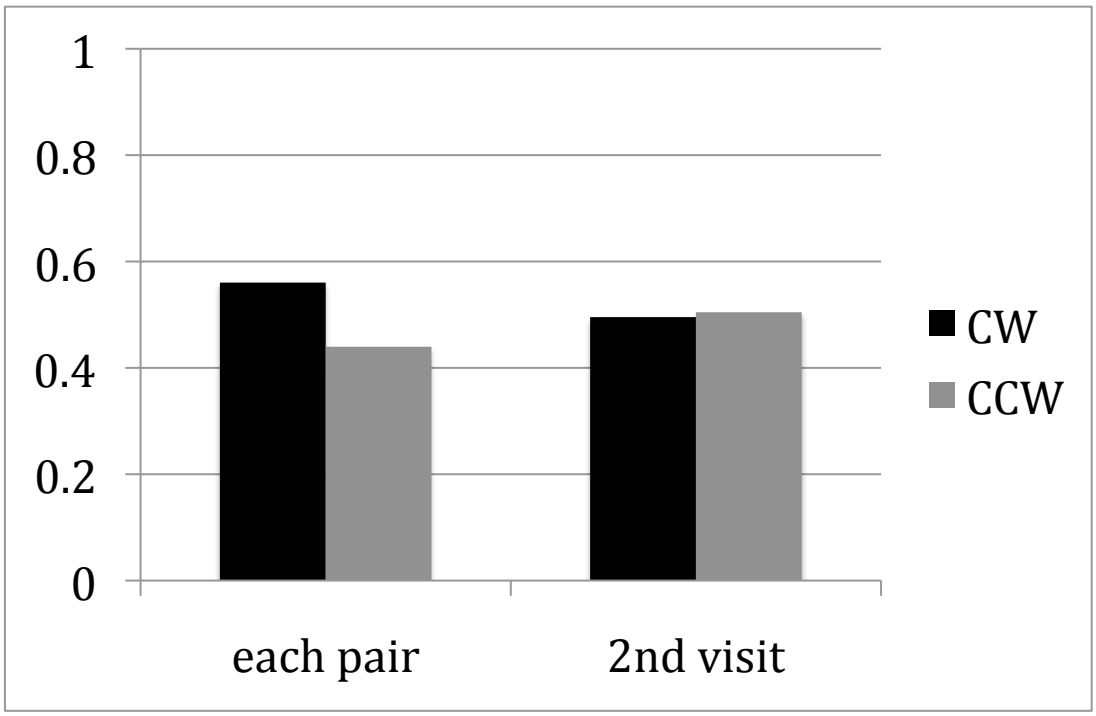
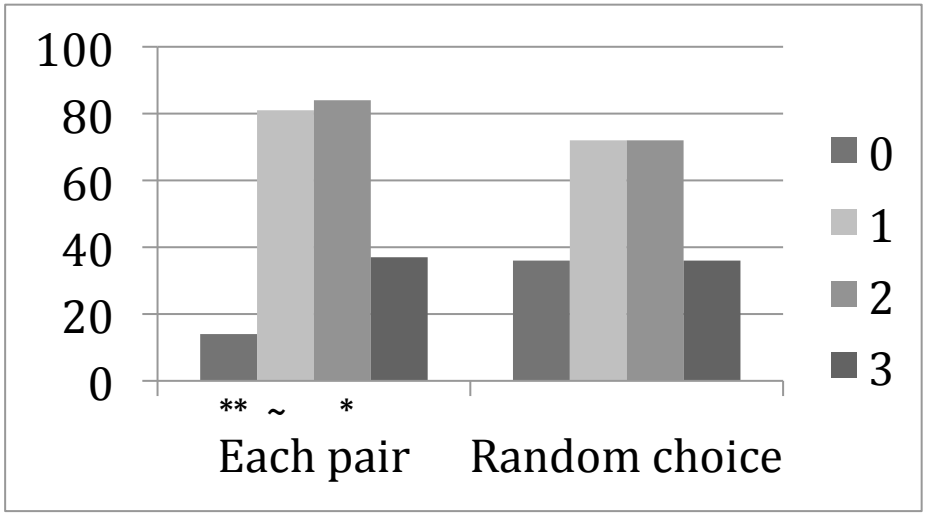


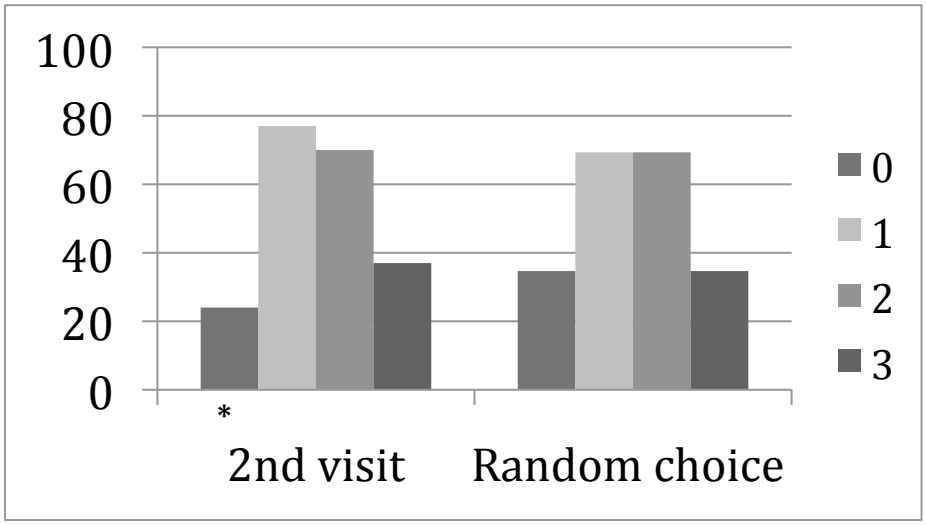
Figure 3. Frequency of clockwise (CW) and counter clockwise (CCW) turns for each subsequent pair (p-value = 0.186) and for the 2nd visit (p-value = 0.804).

Figure 4. Histograms of a) each pair, b) 2nd visit, and c) 3rd visit sequence and random choice. ** significant at 99% level, * significant at 95% level, and ~ significant at 90% level.

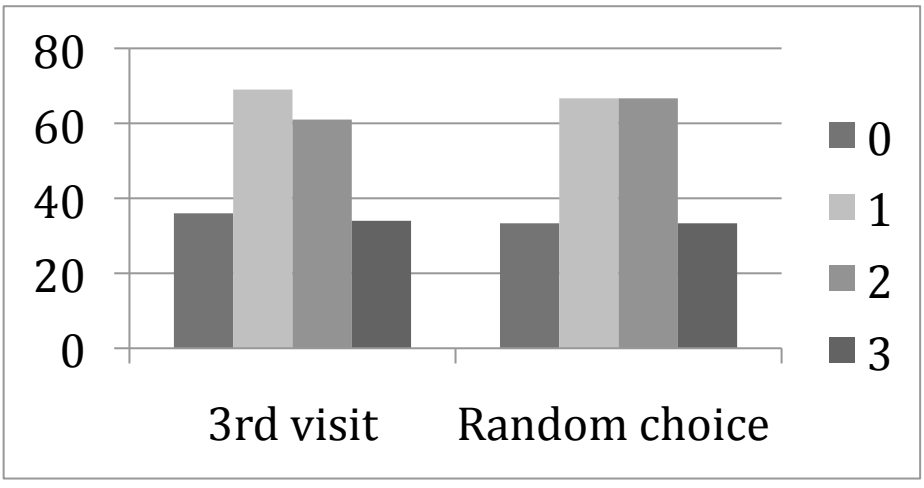
a.


100
80
60
40
20
0
** ~ *
Each pair
Random choice
0
1
2
3

b.


100
80
60
40
20
0
*
2nd visit
Random choice
0
1
2
3

c.


80
60
40
20
0
3rd visit
Random choice
0
1
2
3

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CHAPTER 2: Flexible Preference Between Distal Visual Cues or a Salient, Visual Beacon as Directional Cues for Homing in the House Mouse (*Mus musculus*)

Abstract

Experimental findings with the homing house mouse (*Mus musculus*) corroborate two previously proposed rules about the selective use of spatial perceptual cues that guide rodent navigation in open spaces. First, while still some distance from a goal there is a strong, unlearned predisposition to select distal (background) visual cues over proximal (intra-arena) visual cues, and over odor cues, for controlling the direction of locomotion. Second, this spatial cue selection can be reversed due to repetitive experience with spatially shifting, and hence, unreliable background cues. Specifically, a previously ignored, but spatially stable directional cue—a conspicuous beacon at the goal location—effectively replaces as directional cue the previously preferred, but now ignored, visual background cues. The detailed structure of the learning mechanism required to implement the observed massive changes in selecting spatial perceptual cues is not yet known for any rodent. However, it can be said with certainty that such highly flexible learned cue selection is incompatible with the popular notion of non-discriminating snapshot-learning, as previously mis-applied to such contexts.

Introduction

When a house mouse runs straight to a hidden home from a distance of approximately 0.5 m, it shows the ability to self-locate, i.e., to determine its current location and heading in space, and compute the needed direction and distance home. In previous experiments with an open, circular arena and an almost invisible nest entrance at the periphery, the trained mouse headed home from the arena center by preferentially using the help of distal, extra-arena visual cues rather than proximal, intra-arena cues, or idiothetic input (self-generated cues) provided by the experience of the immediately preceding outward run (Alyan and Jander 1994). Even if an intra-arena, visual beacon marked the nest location, the mouse unexpectedly directed its run home relative to distal cues and not the visual beacon, except when the mouse was very close (20–50 cm) to the beacon (Alyan and Jander, 1997).

As these mice were not trained to prefer one visual cue type or the other among all available cues, their preference indicates an *a priori* inclination to learn and rely on such preferred cues. One question that arises from this preference in selective learning is: is a house mouse trained to locate a goal relative to an enriched visual background capable of learning to ignore these distal cues if they become unreliable predictors of the goal location; and then learn to rely on a different set of visual cues? One persistent and favored theory in spatial cognition, snapshot theory, predicts that these mice cannot learn to selectively ignore the visual background cues. Snapshot theory states that the navigating animal uses a form of image matching to compare remembered 'snapshots' or indiscriminately stored retinal

images, taken when leaving the home base. Thus, to navigate home, self-location involves comparing the perceived current retinal input to the stored images and then the animal returns home by moving until the images visually match (Collett and Cartwright, 1983, Collett and Collett, 2002, Wang and Spelke, 2002, Cheung et al., 2008, Stürzl et al., 2008). If this theory is correct, mice should continue navigating toward the trained direction to find the goal; and would not be able to learn to selectively ignore the visual background images stored in a "snapshot," because a snapshot is an indivisible mental representation.

The purpose of this study is to test this indiscriminate pictorial memory implied by the snapshot theory. First, I will reconfirm the above findings that house mice selectively prefer extra-arena (distal) cues to intra-arena (proximal) cues to control navigating to a known goal. Then, in a second experiment I will use the same configuration of visual cues already experienced by the mice to find out whether the homing mice can learn to selectively ignore the preferred distal cues and instead choose a previously ignored proximal beacon as a directional cue.

Methods

Animals

Seven experimentally naïve, captive-reared, female house mice of mixed strains were used. The mice were fully pigmented (gray to black), with black eyes. They were housed in transparent cages (48 l × 25 w × 15 h cm). Pine shavings and cotton served as nesting material, and food and water were given ad lib. The average age of the female mice was 8 mo., and the litter sizes ranged from 9–14

pups. All mice were kept under a 12:12 h light: dark cycle and were tested during the light phase.

Experimental Setup

The experimental arena was 150 cm in diameter and elevated 70 cm above the room floor. The wooden arena floor was partially covered with cardboard wrapped in light brown paper, for ease of clean up. Plexiglass, 40 cm high, fenced in the arena allowing full view of the diverse room environment. The arena platform rested on a support structure of ball bearings and had accessible handlebars allowing for quick rotation as needed during testing. The periphery of the arena platform was marked every five degrees for the purpose of collecting data on the direction the mice went in the arena. East was designated as 90° in all experiments.

The room was fully illuminated by evenly spaced fluorescent ceiling lights. A vertical black board (60 x 243 cm) was placed 160 cm south from the center of the arena, perpendicular to its north–south axis to serve as a close, conspicuous extra-arena landmark. Various furnishings around the room enriched the visual background.

Nest box

Two identical interchangeable, circular nest boxes were used (fig. 1a). Each box was composed of two fitted parts: a circular wooden platform (16.5 cm in diameter), and a removable circular plastic lid (8 cm tall) with an entrance hole cut in the side. Four vertical wires were attached to the platform to provide an easy method of moving the entire nest box as needed.

General Procedure

Prior to all experiments, a nursing mother and her litter were transferred from their holding cage into the arena, with a nest box placed at the east periphery wall (90°). The mouse had the opportunity to explore the arena for at least one hour before training started. Food and water were always available inside the arena except for the brief testing periods.

Each mouse was trained to navigate from the center of the arena to the east located nest box (fig. 2a). Training entailed moving the full nest box (with mother and pups inside) to the center of the arena, and simultaneously placing the second empty nest box at 90°. Then the lid of the full nest box in the center was removed, leaving the mice exposed and eliciting the mother to retrieve her pups one at a time, back to the peripheral nest box. This process was repeated until each mouse had retrieved at least 100 pups. An upright, black wooden block (8.5 x 9 x 18.5 cm) was placed just to the right side of the peripheral nest box to serve as a beacon throughout training. During training, the experimenter interchanging the nest boxes was visible to the mouse and so periodically changed position, especially at each nest transfer, to avoid becoming a reliable visual cue used by the mouse to orient. Experiment-specific testing followed this general training process.

Experiment 1

The purpose of this experiment was to reconfirm if the mice would use the extra-arena or intra-arena cues to navigate to a learned goal. After training (described in the general procedure) was complete, the mice were tested. Training and testing occurred in one day for each mouse and lasted for about 1 hour, depending on the mouse's motivation to retrieve.

Testing entailed rotating the arena either clockwise or counterclockwise 90°, and leaving the black block, but not the nest box, in the arena to rotate too (so it sat at either 0° or 180°) (Fig. 2b). The full nest box (mouse and her litter) was held above the platform so the mouse would not experience the rotation and then placed in the center of the arena, with no new nest box put in place, and the lid removed. The direction the mouse retrieved her first pup was recorded, within the marked 5° increments. Each datum point was taken only after the mouse walked over a virtual threshold, within 15 cm of the arena periphery, which was where she would encounter the nest box if it were in place. Immediately after the mouse walked to the periphery, the arena was rotated back to its original position and a nest box put in at 90° for the mouse to retrieve the rest of her litter.

Statistics

For the directional data collected, circular statistics were used to compute a mean vector, using the program, Oriana 3. The mean vector is defined by its polar components, μ and r, where μ, is the sample mean direction, r, the length of the mean vector, which is inversely proportional to the variance around μ. This length, r, is reported between 0 and 1, where 1 indicates no variance.

Experiment 2

These seven mice were immediately tested in a second experiment. The purpose of this experiment was to determine if the mice could learn to use a near-nest beacon and ignore extra-arena cues to locate their nest. Training and testing together took one to two hours depending on the mouse's motivation to retrieve.

Using a similar training method to the general procedure, the full and empty nest boxes were interchanged as previously described. However, only 5 pups from the litter were used for retrieval training and each time the empty nest box was placed in the arena it was repositioned among one of four locations at the arena periphery: N, E, S, W. The black block was always placed as a beacon just to the right of the nest (fig. 1b). To avoid repositioning the nest location in a specific sequential pattern the mice could potentially recognize, the position of the nest box was placed in a direction it had not been the previous two placements. For example, if the nest box was placed south, and then at north, the subsequent time could either be east or west.

The nest box was repositioned only after all five pups were retrieved, and thus was recorded as one position change. These methods were repeated around the arena until the mouse could retrieve all five of the pups correctly to the nest box wherever it was placed, for five consecutive nest position changes. The number of nest position changes required for each mouse to achieve this was recorded. It was decided that if the mice were unable to learn to follow the beacon for five consecutive litters after thirty attempts, I would deem that mouse as failing.

Results

Experiment 1

All seven mice walked east toward the trained location with their pup and not toward the rotated location of the beacon at north or south (fig. 3). The mean vector r was 0.99 and μ was 95.7°. All mice picked up their pup and walked directly

to the periphery, which indicates they were not confused when choosing their directional heading.

Experiment 2

Six of the seven mice were successfully trained to locate the nest by correctly using the beacon instead of the extra-arena cues for five continuous litter switches (Table 1). Mouse #4 failed to navigate correctly to the beacon for five consecutive nest switches, but did correctly navigate for four consecutive nest switches. We continued training, but could not elicit five consecutive trials until we ceased training. The average number of trials, of the successful six mice, needed for training before the beacon was used was 15.8 trials and the range was 10–22.

Table 2. Number of training trials until correctly using the beacon to locate nest for each mouse. Mouse indicated with * only completed 4 trials correctly.

Mouse	First correct	Final correct	Cardinal coordinates
1	16	20	S, E, W, N, E
2	22	26	W, E, S, N, W
3	11	15	W, E, N, S, W
4*	17	20	W, E, S, N
5	16	20	E, W, S, N, E
6	10	14	W, E, N, S, W
7	20	24	S, W, N, S, E

Discussion

Main Findings

Experiment 1 was designed to test which type of environmental cue, distal (extra-arena) or proximal (intra-arena), house mice rely on to navigate to a goal after the opportunity to learn both. Specifically, the proximal cues provided were an odor trail and a conspicuous beacon both potentially used to guide the mice to the

nest location. Putting the two cue types in conflict showed the mice fully relied on the distal, extra-arena cues to navigate to the trained location of the nest and ignored the beacon and odor trail (Fig. 2). This result confirms previous studies that found that homing house mice relied on the distal cues and ignored a change in the proximal, intra-arena, landmarks after the opportunity to learn both types during training (Alyan and Jander, 1994).

The mice's preference for using distal cues appears after sufficient experience navigating to a goal; whereas while initially learning to navigate home, mice rely on self-motion cues or a beacon to locate a goal (Alyan and Jander, 1994, Etienne and Jeffery, 2004). During this learning process, the perceived stability of available visual cues is important for an animal to later rely on them for spatial information (Biegler and Morris, 1996).

Adaptive Explanation

Distal cues tend to be larger landmarks because they are visible from a distance, and thus are typically more stable environmental structures, e.g., a large tree. A beacon marking the location of a goal or other more proximal cues is likely smaller and more vulnerable to moving or disappearing over time, e.g., in a storm. Therefore, the *a priori* preference, observed in house mice and golden hamsters, to use distal cues to navigate (Kraemer et al., 1983, Etienne et al., 1990, Alyan and Jander, 1994, Etienne and Jeffery, 2004) could be linked to their stability over time, and is likely instinctive.

Cross-species comparisons

This preference to use distal cues for navigation is found in other vertebrate species too, e.g., rats (*Rattus norvegicus)* (Olton, 1979, Kraemer et al., 1983), and golden hamsters (*Mesocrieetus auratus*) (Etienne et al., 1985). Interestingly, two species of bats, one nectivorous (*Glossophaga soricina*) and one frugivorous species (*Carollia perspicillata*), were inclined to use the more distal cues and ignore beacons, including odor, marking the correct feeder, which is surprising because flowers or fruit often produce an odor to attract the bats (Carter et al., 2010). Scatter-hoarding animals must remember where to relocate their food caches; and many scatter-hoarding birds tested preferred to use distal cues rather than beacons to locate feeders (Herz et al., 1994, Watanabe, 2005, Gould et al., 2010), as did a scatter-hoarding rodent, the fox squirrel, (*Sciurus niger*) (Waisman and Jacobs, 2008).

Parsing local views

The question addressed in the second experiment was if the mice could learn to ignore their strong preference for these distal cues experienced as reliable, if they later become unreliable predictors of where a goal is located. Thus, training attempted to teach the same seven mice to retrieve a group of pups to a nest always marked by a nearby beacon, as both, beacon and nest, were moved together around the arena. Six of the seven mice successfully learned to ignore the distal cues and navigate to the nest box for the (arbitrarily) set limit of 5 consecutive nest position changes. One mouse only navigated correctly to the beacon four consecutive times, and subsequently failed to predict the correct location five consecutive times until

training stopped. When this mouse was incorrect, she was not necessarily heading east using the distal cues, but simply did not correctly locate the goal.

That the mice learned to ignore the distal cues shows flexibility in cue use, determined by those cues that are reliable predictors of a goal location. Some studies on other species show that the use of these distal cues is affected by their reliability. Fox squirrels, while showing a preference for the distal cues, flexibly tend to locate a feeder by searching where the greatest number of cues predict (Waisman and Jacobs, 2008). Gerbils (*Meriones unguiculatus*) also show the inclination to navigate to a location predicted by where the most learned landmarks are in agreement, ignoring an outlying landmark (Collett et al., 1986). Interestingly, scatter-hoarding scrub jays (*Aphelocoma californica*) also relied on distal cues to relocate a food cache unless those cues became unreliable and then a beacon was used (Waisman and Jacobs, 2008).

Theoretical implications

This flexible cue use, and more importantly, the ability of the mice to ignore their strong predisposition to rely on distal visual information already experienced as reliable contradicts the snapshot theory of place learning (Collett and Cartwright, 1983), which implies a holistic mental representation of local views. During this study's second experiment, no attempt was made to disguise or hide the visual background as the mice learned to follow the beacon. Therefore, each time the nest box and beacon were moved around the arena, the visual background of the goal in the foreground was different.

Neuronal circuits

The learning mechanism required to adapt to such a massive change in cue use is not yet known for any rodent. However, recordings from two types of cells in rats, place cells and head direction cells, that can be linked with navigation behavior are sensitive to cue stability, which affects which cue type predominates control over these cell types. Place cells are characterized by firing when an animal is within a specific location (Muller, 1996); and head direction cells fire in response to the direction the animal's head is oriented, independent of location (Taube et al., 1990, Taube, 2007). Both types of cells use both idiothetic and visual information, but visual cues predominate, unless the conflict between the two types of information is too great (Knierim et al., 1998), or the visual cue is viewed as unstable (Jeffery, 1998). Place cells will encode to either proximal or distal environmental cues, but more tend to prefer proximal cues (Knierim, 2002, Yoganarasimha et al., 2006), while head direction cells prefer distal visual cues to proximal cues (Zugaro et al., 2001, Yoganarasimha et al., 2006). Head direction cells showed that when idiothetic and visual cues were in conflict, the visual landmark predominates, unless a cue is repeatedly in conflict with another visual cue already viewed as stable and then this stable cue will maintain the firing direction of the cell (Taube and Burton, 1995). No study has focused on the response of either cell type when an animal is tested in a similar design to this present study, which would provide an opportunity to link the navigation behavior with the cell responses.

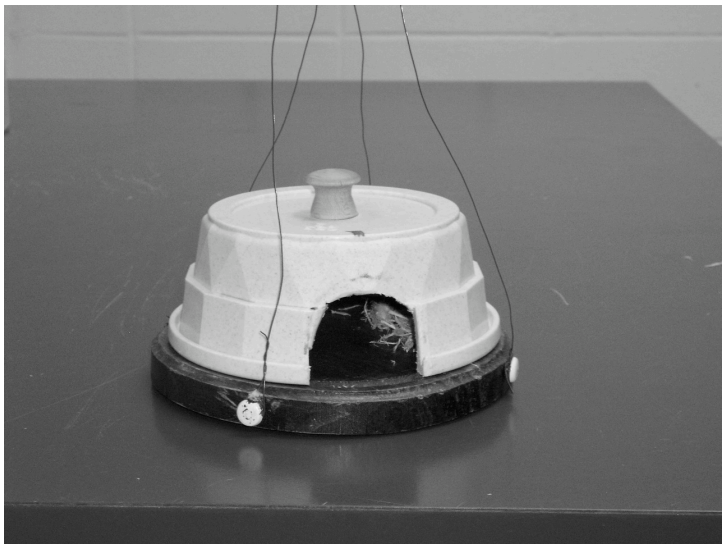
Conclusion

This study first confirmed previous findings that house mice have an *a priori* preference to use distal visual landmarks to navigate to a learned goal, rather than

an odor trail or a beacon close to the goal. Secondly, this study showed that mice are capable of ignoring their strong preference for previously reliable distal cues when they become unreliable, and then learning to use a previously ignored visual beacon as a predictor of the goal location.

Figures

a.



b.

Figure 1.
a) Picture of nest box put together. White plastic lid, with door cut in the side can be removed from the bottom wooden platform. Four wires are attached to the sides of the platform to form a handle above whole nest box.
b) Picture of nest box at periphery of arena with black block upright next to it. This black block served as the visual beacon during Experiment 2.

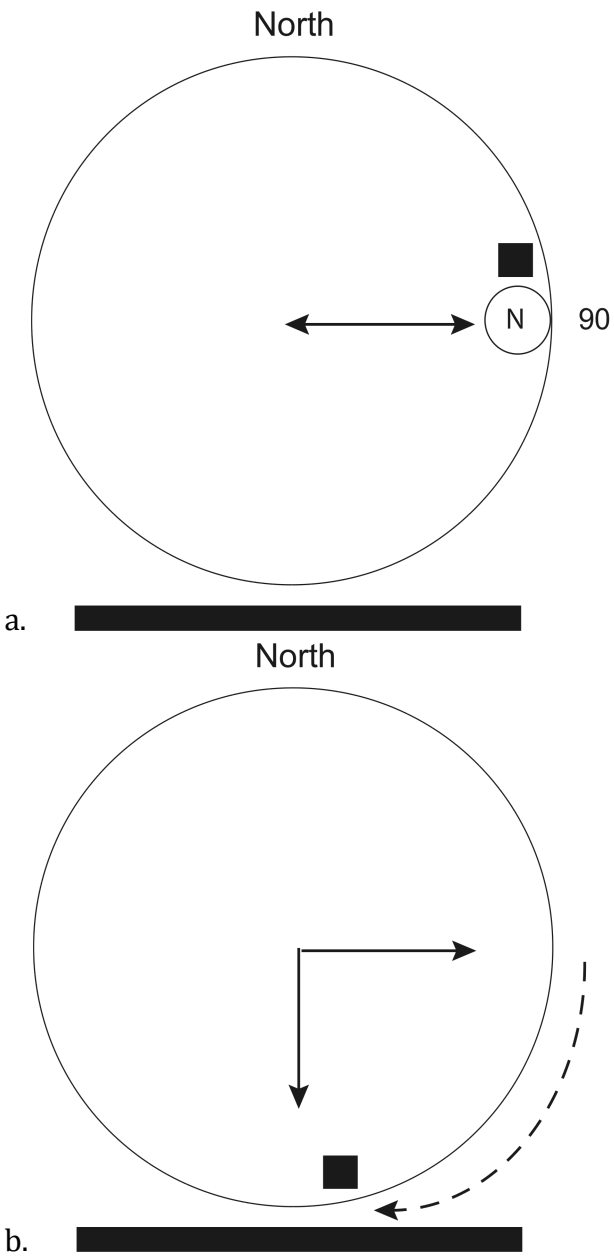


Figure 2. Training (a) and testing (b) set-up for Experiment 1. The solid black rectangle outside of the arena, the circle, represents the black board, the salient extra-arena cue. The black square within the arena represents the black block used as a beacon. Training (a) involved the mouse retrieving pups between the center and the nest, 'N', as shown by the double arrow. During testing (b), the nest box was removed and the arena was rotated either 90° clockwise (as represented by the dashed line) or counterclockwise. The black block remains in the arena and is also rotated. The mouse and litter are place in the center. If the mice use the extra-arena cues, they will walk east. If the mice use the beacon, they will walk toward it depending on the arena rotation.

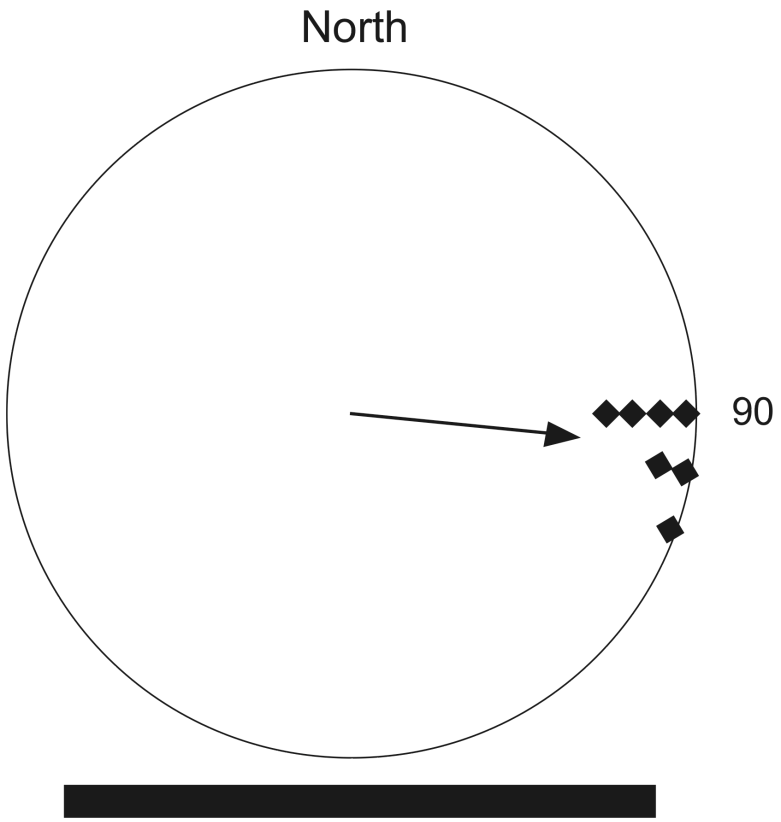


Figure 3. Experiment 1 results. Each diamond represents the direction one mouse walked to the periphery. The arrow represents the mean vector angle. The odor trail and black block have been rotated to either 90° or 270°.

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CHAPTER 3: Feedback control of a navigating house mouse away from home and back: idiothetic or allothetic?

Abstract

An important component of navigating along a well-known route is the animal's ability to confirm that the correct movement is implemented along the way, which occurs via feedback control. Sensory input for feedback control can be idiothetic, allothetic or both. Most past rodent studies testing which cue type predominates directional heading have overlooked the concept of feedback control. These studies report that distal allothetic cues are important for homing rats and mice along a one-way, straight path home, and a few studies have shown mice ignore passive rotation (idiothetic cues) when at home and subsequently leaving on a straight path outward. Thus, rodents may rely on different cue types for navigating on the two parts of a roundtrip, out and home again. In this study, I wanted to find out how feedback control is implemented if the routes to the goal were L-shaped rather than straight. Therefore, I used a plus-maze to train and test house mice to navigate along an L-shaped path out to a goal and then home again. To test, allothetic and idiothetic cues were manipulated by rotating a salient directional light source 90° and/or rotating the arena 90° either during the outward or the homing part of the roundtrip. The results show that mice rely on idiothetic feedback control during the outward path and both allothetic and idiothetic feedback control influence the homing trip.

Introduction

In home range orientation, an animal must be capable of knowing how to get from its current location to a goal in an area it maintains for daily use. A key component for such successful navigation stems from compass orientation—knowing the needed direction to reach a goal—that is maintained through directional information. For directional information, there are two main categories of cues available for rodents: idiothetic and allothetic.

Idiothetic cues—internal cues provided by an animal's own movements—comprise two important types: vestibular and kinesthetic. Vestibular organs provide information about any rotational movement of the body and kinesthetic input provides information about the physical movements taken by the limbs. Allothetic, or environmental, cues are external to the animal's body. Of the allothetic cue types, visual cues are the most important for sighted rodents under lighted conditions (Dunning and Payne, 1979, Etienne et al., 1985, Alyan and Jander, 1994).

Previous studies on homing house mice showed that distal allothetic cues are preferred to local allothetic cues for their heading direction (Alyan and Jander, 1997, Alyan, 2004a). In addition, house mice relied on idiothetic cues for compass orientation rather than visual landmarks while initially learning spatial information about an area (Alyan and Jander, 1994). But after sufficient experience, visual cues predominate over idiothetic cues in house mice, rats and golden hamsters (Kraemer et al., 1983, Etienne et al., 1990, Biegler and Morris, 1993, Alyan and Jander, 1994, Alyan, 2004a). However, if the angular conflict between visual and idiothetic input is

too great (more than 90°) homing directions of golden hamsters seem to rely more on idiothetic input (Etienne et al., 1990).

Knowing the heading to a goal is only part of the process of navigating in a familiar area. Because of error accumulation, it is necessary to correct and confirm, by feedback control, movement along the route to reach the goal. Sensory input for feedback control is allothetic, idiothetic or both (Arleo and Rondi-Reig, 2010). For the above-mentioned studies, straight, unobstructed routes connected the respective start and goal locations, so single-vector feedback control was sufficient.

I wanted to find out how feedback control is implemented if the routes to the goal were L-shaped rather than straight. The need for feedback control might become more obvious when more than one locomotor vector is involved between the start location and the goal, thus the bi-directional trip becomes L-shaped. Specifically I ask which cue predominates the input for the feedback control as the animal decides the direction to walk at an intersection? If idiothetic input predominates, then specifically vestibular input would confirm that the animal's body rotated the correct angle and direction for the turn. Or allothetic cues, likely a salient distal cue, must be perceived to change angle relative to the animal's body to signal a turn has occurred; e.g., a large tree was straight ahead and is now located to the left—implying the animal turned 90°.

The easiest method of testing which of these cue types predominate feedback control for a necessary turn is to use a maze to experimentally manipulate cues while a rodent is negotiating the maze. But in 100 years of maze studies on rodents (for early review see: Tolman et al., 1946a, b, Olton, 1979, Skinner et al., 2003), no

one ever manipulated the self-motion cues (idiothetic) by passively rotating the subject while in the maze, and thus properly testing the effects of vestibular input on directional choice at a maze intersection. What has been shown by maze studies is that rats can be trained to either use allothetic cues or rely on self-motion cues to make the directional choice at the intersection (Tolman et al., 1946b, Skinner et al., 2003), and that if extra-maze (distal allothetic) cues are available rodents will ignore intra-maze (local allothetic) cues when choosing a heading (Honzig, 1936, Kraemer et al., 1983).

Two other important components often overlooked in home range orientation studies are the round trip structure and the home base location. Most animals do not simply travel from an arbitrary starting point to a goal, but rather take a round trip involving an *outward path* from its home out to a goal and then return home via a *homing path*, assuming no other locations are visited on this trip. Typically, published studies focus on a single route taken by the animal—either the outward path to a goal, but usually only the homing path—that leaves an incomplete picture, as it is possible that these two routes require different strategies or different sources of information to navigate. In order to study this more natural home range behavior of a whole roundtrip a home base is required. Specifically, maze studies on rodents typically do not provide a home base, or a nest, but rather have the rodent run through the maze. But all these animals naturally have a home in their home range where they can retreat, and numerous studies report that the first behavior exhibited in a novel, open arena is to adopt a space to serve as a home base (Drai et al., 2001, Avni et al., 2006, Clark et al., 2006, Nemati and Wishaw,

2007). Therefore, providing a nest for the subject rodent is important for learning about more natural navigation; the absence of which may be the reason for ignoring the different components of the whole roundtrip.

The idea that the outward path and homing path involve different navigating strategies is supported by a study showing that house mice, when trained in an open arena to move straight between a nest and a goal, require visual access to distal landmarks to home correctly; but not during the outward path (Alyan, 2004a). Also when house mice trained in a circular open arena to retrieve pups straight from a center goal location to a peripheral nest box experienced a 90° arena rotation while inside their nest, they still left their nest and walked directly out to the center of the arena. However, the mice reoriented relative to the distal landmarks during their subsequent homing path (Alyan and Jander, 1994). If either the idiothetic cues or distal visual landmarks strongly influenced heading on the outward path, the mice should have noticed the 90° rotation and not walked out of the nest back to the center of the arena. These results suggest that both vestibular and visual cues are less important when leaving home than when returning to it.

The purpose of this study was to uncover the navigation strategies of mice negotiating L-shaped, rather than straight, routes away from and then back to home. Specifically, which cues predominate during different parts of more complex round trips? To find answers, I trained house mice to retrieve their pups along an L-shaped route in a plus-shaped maze placed within a circular, open arena that can be rotated (see fig 1). With this design, the mice must choose a directional heading at the intersection to successfully navigate out toward the pups and then home again.

There are several categories of cues in the design of this study that could affect directional choice at the intersection. Local allothetic cues, or intra-maze cues are always available, such as, an odor trail or the walls of the plus-maze; for example, the mice could learn to follow the wall on the right and make a right turn when the maze wall does at the intersection. Another available cue is a salient, distal allothetic cue, which is a directional light source aimed into the arena from one direction during training and shifted by 90° in some tests. Plus, the rest of the visual background will be illuminated by this light source, providing a less salient set of distal allothetic cues that will remain fixed throughout the study. Finally, the idiothetic input will be all self-motion cues from the animal navigating, plus the vestibular input, which can be experimentally manipulated by rotating the arena.

The two cues that were manipulated during a series of experiments in this study are the directional light that is moved 90°, or the passive rotation of the mouse by rotating the arena 90°, or the combination of both. I expected based on past data with straight runs that there would be a difference in which cue predominates during the outward and homing paths. Also, as previous studies on unidirectional navigation suggest, I predicted that the salient visual cue will influence the mice's directional choice the most during the L-shaped homing route.

General Methods

Arena set-up

A plus-maze constructed of plexiglass (4 mm thick) was employed in all experiments (Fig. 1a, b). The walls of the maze were 9.3 cm tall, with the length of three arms at 50 cm and the fourth arm that led to the pups was 69 cm. The width

of each arm was 8 cm. The maze was within an open circular arena (1.5 m diameter), with a transparent plastic sheet around the perimeter (height of 40 cm). The open arena rested on ball bearings for ease of rotation as needed, and its platform was 70 cm above the floor. External to the arena were furnishings in the room that enriched the visual background.

The ends of two arms (north and east) of the plus-maze were walled off with plexiglass. The south arm leading to the pup retrieval site (labeled 'P', Fig. 1a,b) ended at the wall of the larger open arena, and the west arm opened into the nest box (labeled 'N', Fig 1,b). The removable cubic nest box (9 cm wide, 9 cm tall, 12 cm deep) was constructed of plastic with cardboard bedding and was covered in black paper; the only entrance to the nest opened into the maze.

Salient light cue

Two lamps were used as the salient directional environmental cue—only one was on at a time. Both lamps held 60-watt light bulbs aimed into the arena from 1 m away from the open arena periphery, and 50 cm above the level of the arena platform. The lamps were plugged into a switch box so that both lights were controlled by one switch, allowing one lamp to be turned off, and the other on, simultaneously.

General Training Procedure

The training procedure and environment for all experiments was the same, (except for the placement of the light cue). During training, whichever light source was important for that day's experiment was turned on continuously and the overhead room lights off. Then a mouse and her litter were placed in the nest box

from where she was given at least one hour of exploration time before training started. Food and water were always available inside the open arena along the perimeter. All mice were able to climb out of the maze and have access to the food and water ad lib. Each individual mouse was trained and then immediately tested in a series of 3 experiments (either 1–3 or 4–6). The total time for training and testing took 1 to 2 hours, and only one mouse was tested per day.

The mice were all trained to shuttle between the pup retrieval site and the nest (Fig. 1a,b) by taking advantage of the mother mouse's instinctual motivation to retrieve her pups to a safe home base. Therefore, when the litter was placed at the pup retrieval site each mouse would retrieve her pups one at a time back to the nest. To extend the number of pup retrievals beyond one litter, the trainer would wait until all pups were retrieved into the nest and the mouse was out of the nest; then the box was quickly removed from its placement, the pups gently dumped at the retrieval site, and the nest box replaced. This process was repeated until each mouse had retrieved a pup no less than 100 times. At no point in the training or testing were the mice confined to an area or prevented from moving. Therefore, any cue manipulation occurred as quickly as possible during the appropriate time of the outward or homing path (described in the following descriptions for each specific experiment).

Special Methods and Results

Experiments 1–3: Testing outward path navigation

Experiments 1–3 centered on the mice's use of cues on their outward path toward the pup retrieval site (goal). For these experiments during training the light

cue was directed into the maze from the south. Therefore, the training objective was for the mice to walk out of the nest and at the intersection orient toward the light to find the pups (Fig. 1a). Each of 10 individual mice was given one training session, and then the following three experimental testing procedures occurred in a series.

Experiment 1: Light cue change, south to east

To determine the effect of the salient light cue on directional choice during navigation in the maze, the light source was instantly changed from the south lamp to the east lamp while the mouse was walking out of the nest box towards the pups (Fig. 2a). This cue switch occurred while the mouse was still in the west arm of the maze—before the intersection. Each mouse had three options at the intersection when the light was changed: 1) to follow the light cue, and walk straight (east), 2) to follow the idiothetic input and turn right, as trained or 3) turn left (north) at the intersection in confusion.

Experiment 1: Results

When the light cue was shifted from south to east, all 10 mice kept walking south at the intersection (Fig. 2a). The mice systematically turning right at the intersection, as they had done during training (Fig. 2a solid arrow), implies the light played no major role, if any, in the mice's directional choice at the intersection.

Experiment 2: Vestibular information affected while mouse outside the nest

While each mouse was *leaving* the nest box (still within the west arm, but not inside the nest box), the entire arena was quickly, and smoothly rotated 90° clockwise (CW), so that now the pups were located at the west arm, and the nest in

the north (Fig. 2b). The light cue remained south. If the mice take into account the idiothetic input experienced by the rotation, they should respond at the intersection as if they already turned and head accordingly (straight) to the *absolute location* of the goal, where it had previously been located. If the mice ignore the experienced rotation and continue along the trained route, then the mice will continue to turn right at the intersection and head to the *relative location* of the pup retrieval site now rotated. Thus, the following three options could occur: 1) the mice take into account the experienced rotation (and/or follow all allothetic cues) and proceed south, 2) the mice ignore all cue changes and turn as trained, right (west) to the pups, or 3) the mice turn left (east) in confusion.

Experiment 2: Results

After the arena was rotated, 6 mice walked straight (south) at the intersection, 3 mice turned right in the trained direction, which was now west, and 1 mouse walked east (Fig. 2b). The two null hypotheses tested for this experiment were: 1) The rotation has no effect and the mice will continue turning right, as trained, at the intersection; and 2) There is no difference in the number of mice who chose to walk straight (toward the absolute goal location) or to turn right (toward the relative goal location). The first null hypothesis that all mice would continue as trained and turn right, ignoring the passive rotation, can be rejected. Thus, the mice have a tendency to go either to the relative or absolute location of the pup retrieval site. Using a binomial distribution statistic, the second null hypothesis cannot be rejected (p-value = 0.51), but the data showed the tendency to go straight (absolute location), rather than to turn right, was stronger but not significant.

The cues linked with walking straight at the intersection were the vestibular input, and the distal allothetic cues; the local allothetic cues were ignored. In contrast, mice that turned right to find the pups were possibly relying on local allothetic cues, e.g., odor trail, and ignoring the vestibular input and all distal allothetic cues.

Experiment 3: Vestibular information changed while inside the nest

This experiment was to determine the effect of passively rotating the arena, while the mouse was inside the nest, on the navigating mice's directional choice to the goal. Similar to Exp. 2, the arena was rotated 90° CW and the light cue remained south while the mouse was *inside the nest box* (Fig. 2c). The same three options were available as listed in Exp. 2.

Experiment 3: Results

All 10 mice turned right (west) at the intersection (Fig. 2c). Thus none of the mice used the rotated allothetic cues nor took into account the experienced idiothetic input during the rotation and walked straight (south) at the intersection (Fig. 2c dashed arrow), but instead continued to the relative location of the pup retrieval site. These results imply that the mice ignored the vestibular input and all distal allothetic cue changes, but this did not eliminate their use of local allothetic cues while navigating out of the nest.

Experiments 4–6: Homing path navigation

The next 3 series of experiments focused on the homing path of the mice from the pup retrieval site (goal) to the nest box. Therefore, the manipulations for each test occurred while the mouse was at or near the goal location. Similar to the

first series, one mouse (of 10 mice total) was trained in a day and then a series of 3 experiments immediately followed. Also, the training procedure and the nest and goal locations were the same as in the first 3 experiments, but the light cue was directed into the maze from the east side of the arena (Fig. 1b). Therefore, the training focused on the mice learning to get home from the pup retrieval site by orienting so the light was behind them (or to turn left in the maze). All cue manipulations occurred when each mouse was at the pup retrieval site at the end of the south arm.

Experiment 4: Light cue change, east to south

To determine if the light influenced the heading of the mice navigating home, the light cue was switched instantaneously from the east to the south while each mouse is at the pup retrieval site. The three possible outcomes the mice could take were (Fig. 2d): 1) the mouse orients to the light and goes straight (north) at the intersection, 2) the mouse continues to turn left (west) at the intersection as trained, or 3) the mouse is confused and goes to the right (east).

Experiment 4: Results

After the light cue change, 4 mice turned left in the trained direction (west) 3 mice walked straight, north and 3 mice turned east (Fig. 2d). This random spread of data implies that the mice did not ignore the light cue change, but were not exclusively relying on the light cue for directional input. Perhaps the mismatch in the salient distal allothetic cue with the other distal allothetic cues and idiothetic input disturbed the homing mice's compass orientation. Therefore the light was an

element in the overall expected stimulus complex that when moved, disturbed the mouse into confusion.

Experiment 5: Vestibular information changed before going home

To determine the effect of passive rotation of the arena on homing navigation, the arena was rotated 90° counterclockwise (CCW), but the light cue remained at the trained east position (Fig. 2e). Thus the pup retrieval site was located in the east arm and the nest box was south. The three possible outcomes for this experiment were: 1) the mice took the rotation into account (and/or followed all allothetic cues) by going straight (west) at the intersection toward the absolute nest location, 2) the mice ignore the rotation and turn left (south) at the intersection to the relative nest location, or 3) the mice turned right (north) in confusion.

Experiment 5: Results

When the arena was rotated 90° CCW while each mouse was at the pup location, 9 mice walked straight (west) at the intersection and 1 mouse turned left (south) (Fig. 2e). The null hypothesis that the mice would ignore the rotation and continue turning left at the intersection can be rejected. Also, using a binomial distribution statistic I rejected the null hypothesis that there is no difference between the absolute and relative nest locations (p-value = 0.02). Thus the mice significantly took the rotation into account and walked straight toward the absolute location of the nest (Fig. 2e dashed arrow). These results imply the mice ignored local allothetic cues and relied on the vestibular input and/or the distal allothetic cues to choose a heading home.

Experiment 6: External and internal cues are in conflict

For this experiment, before the mice walked home, the light cue was moved from east to south at the same time the arena was rotated 90° CCW, putting the two cues in conflict (Fig. 2f). This rotation again puts the pup retrieval site at the east side and the nest box at south. The three possible directional options for the mice were: 1) to take the rotation into account (or use the less salient allothetic cues) and walk straight (west) at the intersection, 2) to orient to the light cue and turn right (north) at the intersection, or 3) to turn left (south) as in training.

Experiment 6: Results

On their way back to the nest, 8 mice walked straight (west), 1 mouse turned right (north), and 1 mouse turned left (south) (Fig. 2f). The null hypothesis that all mice would continue turning left (south) as they were trained can be rejected. A binomial test between the other two directions shows that the mice significantly walked straight rather than following the light cue (p-value of 0.039). Therefore, the mice significantly chose to walk straight at the intersection (Fig. 2f, dashed arrow west), rather than follow the light cue and head north, or to turn left at the intersection as trained. Kinesthetic input can be eliminated as a source of information for feedback control, because otherwise the mice would follow the same walking pattern every trip. This choice to walk straight shows that idiothetic input and/or the less salient, distal allothetic cues (not the light) predominated the mice's homing path.

Discussion

The major questions asked in this study were first, how the mice confirmed the 90° turn they were trained to make halfway toward the goal; and second,

whether there was a difference in cue influence between the outward and the homeward part of the roundtrip. In sum, what was discovered was that for the outward path, vestibular input affected the directional choice of mice at the intersection only while already navigating along the route, and the salient light cue was not important. But for the homing path, a more inclusive stimulus pattern is used; vestibular input is still important, but distal allothetic cues also play a role. Therefore, there was a difference between the outward and homing path navigational strategies, but my prediction was incorrect that the salient light cue would predominate the homing trip. The following sections will discuss more specific outcomes from the series of experiments.

Outward path

The results of these three experiments combined provide evidence that the light cue, and likely all distal allothetic cues, are not important for feedback control during the outward path (Exp. 1, Fig. 2a), which confirms a previous study with straight runs that showed mice do not require visual access to distal landmarks during their outward path (Alyan, 2004a). Also, vestibular input for feedback control affected the mice's directional choice at the intersection, while en route (Exp. 2, Fig. 2b), otherwise any cue changes that occur before leaving home were ignored (Exp. 3, Fig. 2c). That is, the mice in Exp. 3 ignored the vestibular input from the rotation, while in the nest, and the rotated distal allothetic cues, which would be perceived upon leaving the nest, to continue as trained to turn right. Although, the results from Exp. 3 cannot rule out the mice relied on local allothetic (intra-maze) cues to navigate to the goal, the use of these cues was unlikely as numerous studies

have shown that sighted rodents prefer distal (extra-maze) to local (intra-maze) allothetic cues for directional information (Honzig, 1936, Dunning and Payne, 1979, Kraemer et al., 1983, Alyan and Jander, 1994), including chapters 2 and 4 in this dissertation.

A previous study in which mice were trained to shuttle along a straight path between their nest and a distal goal also showed that mice ignored directional cue changes while at home and subsequently exited (Alyan and Jander, 1994). These mice ignored the rotation experienced within an open arena while they were in their nest box and instead showed a tendency to leave the nest and walk toward the center of the arena.

Thus it appears that the mice completely ignored any allothetic cues during the outward path, and instead relied on vestibular feedback control while en route. Why the difference between Exp. 2 and 3? While already walking along the route out of the nest and experiencing the passive rotation (Exp. 2), the feedback control from the vestibular input confirmed a right turn occurred and so the motivation to turn right at the intersection was eliminated. However, if this passive rotation is experienced while inside the nest prior to departure, this passive rotation was ignored and the motivation to turn right at the intersection remained intact.

Homing path

When the mice were homing, manipulating the salient directional light cue (Exp. 4) resulted in random orientation (Fig. 2d). This randomness indicates that the change in light cue caused a disturbance in the inclusive stimulus pattern relied on for feedback control during homing. Although the light was not the predominate

cue used, it was a critical constituent of the whole local view shift. The results from the light cue change support previous findings that distal allothetic cues are important for unidirectional homing (Kraemer et al., 1983, Etienne et al., 1985, Etienne et al., 1990, Alyan and Jander, 1994, Alyan, 2004a).

Similar to the outward path, vestibular input, while en route home, plays an important role in directional choice (Exp. 5), as the passive rotation caused a significant tendency to walk straight at the intersection (Fig. 2e). Therefore, intra-maze allothetic cues are not important for homing. When the arena was rotated, the vestibular input change matched all distal allothetic cue changes, so which cue type predominated cannot be decided with this outcome. When both, the light cue and the arena were rotated to be in conflict, mice significantly walked straight at the intersection, taking the rotation into account and ignoring the light (Fig. 2f). Thus the light did not have as strong an effect as the vestibular input, which contradicts previous findings in behavioral studies showing that use of salient visual cues predominated a straight homebound trip (Kraemer et al., 1983, Etienne et al., 1985, Alyan and Jander, 1994, Etienne et al., 1996, Alyan, 2004a).

Independent evidence for the dominating role of the distal allothetic directional cues for homing come from neurophysiological studies on head direction cells. These cells, found in the rat hippocampal complex, can be linked with the behavior associated with compass orientation. Head direction cells have a peak firing response when the animal's head is oriented toward a direction specific to a cell (Taube, 2007). Both idiothetic and visual information control the firing of these cells, but salient visual cues predominate influence over directional firing (Jeffery,

1998, Zugaro et al., 2001, Yoganarasimha et al., 2006, Taube, 2007), unless the conflict between the two is large (greater than 135°)(Knierim et al., 1998).

Typically in previous studies, to change an environmental cue, the subject animal was confined or covered while the cue used (or another visual landmark) is moved. There is evidence suggesting that over time, e.g., while the animal is confined so the visual cue card can be moved in the experiment, idiothetic input degrades (Jeffery and O'Keefe, 1999). So in my experiments, the instantaneous switch of the light meant that the manipulation could occur with minimal interference in the animal's movement and no degradation of the working memory. Eliminating the time needed to confine the animal could account for the predominance of idiothetic input for feedback control during the L-shaped homing route observed.

Alternatively, the results could be explained if the mice used available environmental background cues, instead of the light, for directional information. Although the directional light source was the most salient, it did illuminate part of the enriched visual background external to the open arena. Mice learned to navigate a unidirectional path using this visual background and ignoring the salient light cue when it became unreliable (Pass and Clifton, unpublished data), so perhaps some mice were using the less salient visual cues for directional information in this study. There are no studies that focus on what type of visual cue is used specifically for directional information. Also, there are very few studies that focus on rodents' use of directional light for compass orientation, which I will discuss in the next section.

Another possibility is that the house mice, in Exp. 6, ignored the salient light cue because it did not conform with the shift of both the idiothetic input and all other distal allothetic cues. When the arena was rotated, the vestibular input was still matched with the less salient allothetic cues. Previous rodent studies on fox squirrels (Waisman and Jacobs, 2008) and gerbils (Collett et al., 1986) have shown a flexible cue use with a tendency to search for a location where the greatest number of allothetic cues predict, ignoring an outlying landmark—even if that outlier is the preferred cue. House mice may also have this flexible cue use for homing, as discussed in Chapter 2, and so the more cues that match (i.e., vestibular input and all the less salient, distal allothetic cues) may predominate the mismatched salient, distal allothetic cue. Thus further studies on homing should consider that mice may have flexible cue use for feedback control while navigating a known route.

Compass orientation via directional light

Most rodent studies testing compass orientation leave one environmental cue, typically a colored cue card, available in the design. In nature, the sun is a prominent cue available for compass orientation in homing animals, and female meadow voles use it for compass orientation (Fluharty et al., 1976). In the lab, experienced homing house mice tested in an open arena rely on extra-arena (distal) cues to navigate home via a unidirectional trip, but when the salient environmental cue is a light source, moving it 90° had no effect on homing (Alyan and Jander, 1994). However Pass, et al. (unpublished data) showed that moving the salient light cue 180° around an open arena caused the homing mice to reverse direction, unless trained to ignore the light. Also, the homing path of golden hamsters in an open

arena is influenced by a dim directional light cue (Etienne et al., 1990). These seemingly conflicting results highlight the need for further studies on the variable effect of light cues on rodent navigation.

Feedback control and previous studies

The important concept of feedback control is often overlooked as a cause for observed behavioral responses in maze studies. Previous maze studies have shown that rats can be trained to locate a goal along a L-shaped route by always turning right (or left) regardless of starting position in a maze or an open arena (Tolman et al., 1946b, Skinner et al., 2003, Stringer et al., 2005). Although it is important to note that no nest was provided in the maze so the tested route was neither outward nor homing. The authors called this behavior to always turn right (or left) "response learning," which is confusing because no one ever identified the hypothetical stimulus to which the "response" behavior was responding. In these same experiments other rats were successfully trained to navigate using an allothetic cue to locate a goal in a specific compass direction (e.g., north) too, which were called the "directional response" group (Skinner et al., 2003, Stringer et al., 2005). These rats could only learn to navigate this route after sufficient training. Therefore, the route was an established route by the time testing occurred, but the concept of feedback control guiding the rats' directional decisions at the maze intersection was not considered.

The concept of feedback control easily explains the behavioral differences of these "directional" and "response" groups of mice, as it seems apparent they were specifically trained to rely solely on allothetic input or only on idiothetic input to

confirm a 90° turn was executed to reach the goal. One group of mice was trained to turn toward a visual cue, and so always headed in a specific compass direction (the "directional learning" group) and the other group relied on idiothetic input to confirm the 90° turn was executed correctly for turning right (or left) at the maze intersection (the "response learning" group). Had the maze been passively rotated during the experiments, undoubtedly vestibular feedback control would have affected the rats' behavioral navigation response, just as seen in this study.

Theoretical implications

Why would a navigating mouse ignore allothetic cues during the outward path? In a natural setting, a mouse would spend proportionally more time at its home base (Drai et al., 2001, Avni et al., 2006, Clark et al., 2006, Nemati and Wishaw, 2007), presumably a safe nest area. The environment for the outward path may have changed if enough time elapsed after returning home. Therefore, the mouse cannot rely on the landmarks, but it can rely on the locomotor pattern learned from numerous trips out to the goal from home. So when preparing to leave the nest, toward a well-known goal along a route previously traveled, the mouse pre-forms the route mentally and relies on vestibular feedback control to confirm the correct execution. This strategy is less affected by any changes to the environment since the last journey outside.

However during a longer trip, navigational errors are bound to accumulate. Hence error correction with the help of allothetic cues becomes increasingly important with time away from home. Also, an animal may travel around to more than one location before heading home, so using allothetic cues for directional

information is useful. And an inclusive stimulus pattern using multiple sources of directional information seems more reliable than relying on one cue.

Main Conclusion

This study trained mice to navigate a L-shaped route out to a goal and then home again, finding that mice use different navigation strategies during the two parts of a round trip. The mice followed a pre-formed mental route out toward a goal by relying on vestibular feedback control while en route, simultaneously ignoring all cue changes happening while at home. By contrast, both vestibular input and distal allothetic cues affected the directional choice at the maze intersection. The use of a salient directional light source as one of these compass cues to home seems to be less important than idiothetic input and/or less salient distal allothetic cues.

Figures

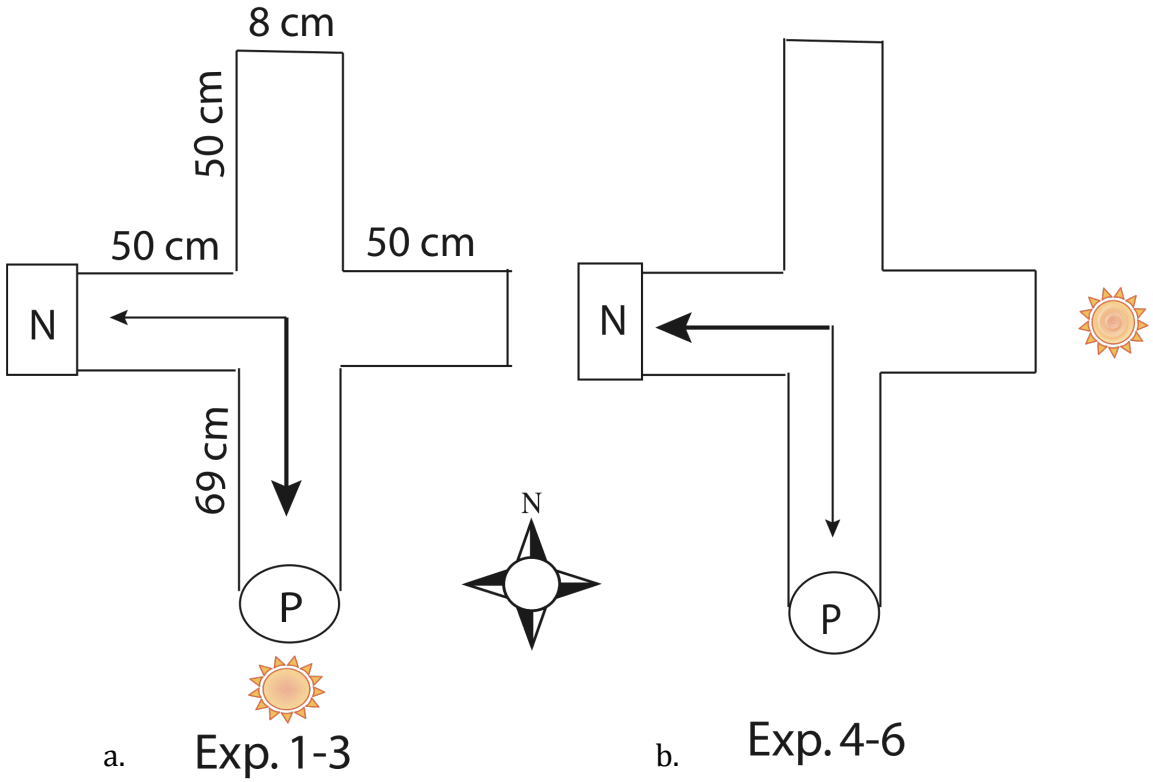

Figure 1. Training protocol used for experimental series 1–3 and 4–6. In all training mice were trained to retrieve pups in a plus-maze, with the pups (P) located at the end of the south arm, and the nest (N) at the end of the west arm. All mice would walk between the two locations, retrieving pups one at a time. In Exp. 1–3 the mice were trained to find the pups by walking out of the nest and turning right at the intersection heading toward the light (south). For Exp. 4–6, the mice were trained to walk home from the pups by turning left at the intersection and walking away from the light source (east).

Figure 2. Results from Exp. 1–6. The arced arrow represents the direction of 90° rotation of either the light or arena. The dashed rectangle shows where the mouse was during the rotation. The straight arrows within the maze show the direction the mice would take if they continue along the trained trajectory, whereas the dashed straight arrows represent the direction the mice would go to follow either the light cue or idiothetic cues. The numbers at the end of each arm show the number of mice that chose to walk into a particular arm during trial 1 and trial 2 is represented in parentheses.

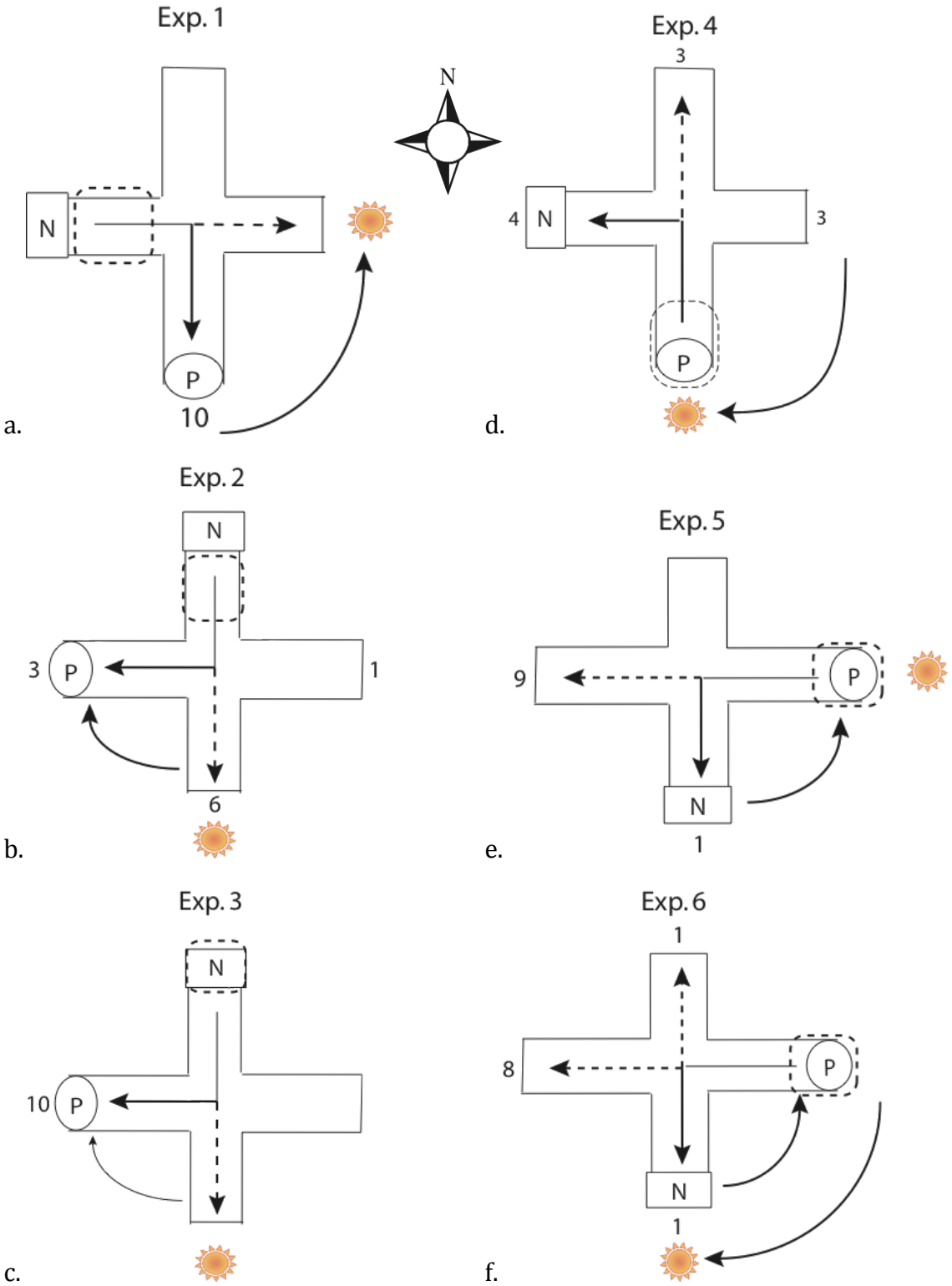
Exp. 1
N
N
P
10
a.
Exp. 4
3
4
N
3
P
d.
Exp. 2
N
3
P
1
6
b.
Exp. 5
9
P
N
1
e.
Exp. 3
N
10
P
c.
Exp. 6
1
8
P
N
1
f.

Figure 2

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CHAPTER 4: The Shortcut and the Map in the Rodent Navigation System: Experiments with the House Mouse (*Mus musculus*)

Abstract

The concept of a cognitive map states that an animal can deduce a novel shortcut to a goal by using only a map-based computation. Past studies have unsuccessfully tested rodents for this map-based shortcutting by failing to design an experiment that excludes all other forms of novel shortcutting, e.g., path integration. In this study, I propose a testable hypothesis called viewpoint extrapolation that refers to an animal's ability to use three-dimensional spatial information known from one perspective to a known goal and to extrapolate that information to a novel place for successful navigation back to the goal. My experimental design trained female house mice in a circular, open arena (1.5 m diameter), with full visual access to the surroundings, to retrieve their pups from the center to a nest box (goal) at the north periphery. I then shifted the arena by its diameter directly north and tested the mice's ability to navigate back to the goal from the center of this new position. In the displaced position, the data showed increased scatter but a slight directional trend north. I interpret these results to indicate that the compass orientation was not place specific, but the mouse failed to reorient relative to the original location of its nest, which she could have done by viewpoint extrapolation. A second experiment was used to determine if these two positions were far enough apart for the mouse to distinguish between them. After training in the second experiment, the mice successfully discriminated between the two previously undistinguished places. These experiments prove that the house mouse failed to show the use of viewpoint

extrapolation to navigate, and so was unable to compute a novel map-based shortcut home.

Introduction

A century of research establishes that rodents are excellent navigators, implying that they are apt to move toward hidden goals with great efficiency. Such efficiency is supported by their ability to discover novel shortcuts to a goal, if their path is not yet straight. A half a century ago, two models had been advanced independently to explain shortcutting: the "cognitive map" for rodents (Tolman, 1948) and "path integration" for ants (Formicidae) (Jander, 1957). Then a quarter century later, Mittelstaedt and Mittelstaedt (1980) were the first to demonstrate path integration in rodents.

Unfortunately, Tolman (1948) failed to define precisely his concept of a cognitive map, nor did he elaborate on the deductive mechanism that derives shortcuts from map knowledge. These omissions, together with the great appeal of Tolman's map, created controversies and conceptual chaos that has lasted to this day (O'Keefe and Nadel, 1978, Morris, 1981, Poucet, 1993, Benhamou, 1996, Eichenbaum et al., 1999). This state of affairs renders Tolman's concept of a cognitive map virtually useless. On the other hand, the idea of path integration—the computation of a shortcut vector from the locomotor elements of detours—was originally well substantiated and defined. Indeed, "path integration" is now used as an explanatory concept for navigation abilities in hundreds of publications with a high level of consensus (Mittelstaedt and Mittelstaedt, 1980, Etienne and Jeffery, 2004).

Given this history, here I tackle two problems, one theoretically and one experimentally. How to best resolve the conceptual chaos generated by Tolman's

"cognitive map"? And can I find explicit evidence for map-based, novel shortcutting that cannot be alternatively explained by path integration?

I can think of only three distinct ways an animal in its home range can find a shortcut to some goal point without searching for it: one is by guidance, and the other two are by some navigation mechanism. First, an animal can directly perceive the goal itself or some beacon nearby, or use the guidance of an odor trail toward the goal. Second, an animal can use path integration, as has been sufficiently documented for rodents and a number of other animal taxa (Collett and Collett, 2000, Etienne and Jeffery, 2004). Third, I propose the hypothetical possibility of a testable, purely map-based shortcutting method, which can then be tested with the house mouse (*Mus musculus*). Apparently, this has never been done before explicitly. Importantly, such a shortcut mechanism must clearly differ from the other two mechanisms mentioned above. This third mechanism I call shortcutting by means of *viewpoint extrapolation*.

Viewpoint extrapolation requires two related faculties. First, the subject must use its three-dimensional vision to map its first location with respect to the distances and directions of a set of terrestrial cues, such as landmarks. Second, when displaced without access to active or passive path integration to a novel location from where some or all of the previously mapped cues are still visible, then the subject can use viewpoint extrapolation to update its current location, or self-locate, and infer the novel shortcut to the previously mapped, but invisible, first location (Fig. 1).

The purpose of this study is to appropriately test whether the house mouse, as a model species, is capable of finding a shortcut by nothing but viewpoint extrapolation. I trained mice to navigate to a goal in an open arena, and then shift the arena by its diameter leaving no overlap in space to test their ability at the novel location to find the direct shortcut back to the goal.

General Methods

Animals

I used experimentally naïve, captive-reared, brown to black (and 1 white) female house mice (sample size varied by experiment). They were raised in transparent cages (48 l × 25 w × 15 h cm). Pine shavings and cotton were used as nesting materials, and food and water were given *ad lib*. The average age of the mother mice was 8 mo., and the litter sizes ranged from 6–15 pups. All mice were kept under a 12:12 h light:dark cycle and were tested during the light phase.

Arena

Throughout, I used the same circular arena (1.5 m diameter) with a sealed wooden floor, partially covered by cardboard wrapped in light brown paper. A transparent plastic sheet walled off the arena to a height of 40 cm. The edge of the arena floor was marked at 5˚ intervals to facilitate collection of directional data. The arena rested on a support structure of ball bearings and wheeled legs 70 cm above the floor of the room. This support permitted quick, smooth rotation, and translation of the arena as required by the following experimental procedures.

Nest box

Two identical nest boxes (for a picture refer to Chap. 2, fig. 1a) were interchanged as needed throughout training and testing. Each box was composed of two fitted parts: a circular wooden base plate (5 cm in diameter), and a removable circular white, plastic lid (8 cm height with both parts), with a hole in the side to serve as an entrance. Four vertical wires attached to the base plate, and joined above the nest lid, made a handle to move the nest boxes as needed.

Outside the arena

The room was fully illuminated by evenly spaced fluorescent ceiling lights, and furnishings enriched the visual background. In addition, a tall blackboard (60 × 243 cm) placed tangentially to the arena 10 cm away from the south periphery served as a close, conspicuous landmark.

Experiment 1: Methods

The purpose of the first experiment was to determine the degree to which an experienced mouse can extrapolate spatial knowledge acquired in one explored place to another unexplored, nearby place, from where all the same landmarks were visible, but from a different perspective (Fig. 2a). In brief, do mice have the ability to use viewpoint extrapolation to navigate to a previously approached goal?

Each of 30 mice were individually trained, given a visual acuity test, followed by a probe test, which will be referred to as the *pre-translation test*. Then each mouse was tested for the ability to navigate via viewpoint extrapolation, after the arena was shifted to a new location; this test will be referred to as the *post-translation test*. Within each test for each mouse, two separate test *trials* were performed (labeled Trial 1 and 2); the second trial served only to observe the

consistency of behavior. This sequence of training and testing occurred in one day, one mouse per day, and totaled between 1.5 and 2.5 hours, depending on activity level of a mouse. All directional data was recorded within the 5° bins, as the arena was marked. The following sections will describe each event in the sequence.

Training Procedure

The nest box was placed in the arena at the north periphery (0°), with a small amount of nest material inside the box so that the mouse readily accepted it as her home base, into which she could retreat and instinctually retrieve her pups. The mouse and litter were placed in the arena 1 hr before training began, giving the mouse time to explore freely. Food pellets and water were always available inside the arena, placed at the periphery around 90°.

The north position of the nest box served as the goal location the mice were trained to relocate, motivated by their instinctual urge to retrieve their pups to a safe place. Therefore, training involved exposing the pups in a release site at the center of the arena, and the mother mouse would then continually retrieve the pups back to the nest. Training involved the following three-part procedure (using the two identical nest boxes): (1) the full nest box, with the mouse and litter were inside, was transferred from the home-base location to the release site at the center of the arena, where the plastic lid was removed; (2) simultaneously, the empty nest box was placed at the original north nest location; and (3) the exposed mouse retrieved her pups, one by one, from the center release site to the nest box, which was the goal location. This three-step procedure was repeated until each mouse had completed at least 100 pup retrievals from the center to the nest box at 0° in the

arena periphery. Throughout training, the experimenter stood at random places around the room in order to prevent becoming a reliable visual compass cue used by the mice.

Testing Visual Attention

Because vision is important for an animal capable of viewpoint extrapolation, this experiment necessitated all mice were sighted; therefore at some point during training, the mice were all tested for visual responsiveness. This vision test involved temporarily placing a black wooden block (8.5 × 9 cm and 18.5 cm tall) upright in the arena while the mouse was inside the nest box. If this novel visual stimulus elicited approach and close exploration, the mouse was judged to be sighted. The few mice that did not respond to the wooden block were not used for any further testing in this study.

Pre-translation test

After training was completed, each mouse was given two trials of the pre-translation test. This test served three important purposes: (1) as a probe to ensure that training each mouse to navigate to the north goal location was successful; (2) to determine whether the mouse, while homing from the center to the periphery of the arena, made use of intra-arena and/or extra-arena directional visual cues; and (3) as a control data set to compare to the post-translation test to follow.

Initially, this test involved transferring the full nest box to the center of the arena, removing the lid and *not* placing an empty nest box in the arena. Also, the water dish and food were removed. If the mother did not take the pup within 30° of where the nest box should be (0°), the mouse was trained with at least 30 more

retrievals until she passed. The heading direction was recorded only after the mouse walked within 15 cm of the arena periphery. Both the error angle of ±30° and the virtual threshold of 15 cm were designated because they matched the range of variables the mouse would have encountered the nest box had it been in place.

After the first nine mice were trained and tested, this pre-translation test was adjusted by adding an arena rotation for the subsequent 21 mice tested. The arena was rotated either 90° clockwise (CW) or counter clockwise (CCW) just before placing the full nest box in the center release site and removing the lid, to control for all intra-arena cues (e.g., odor trail). Immediately following the first pup retrieval, the arena was rotated back to its original orientation and an empty nest box was again placed at 0° for the remaining pup retrievals in the litter. The test was repeated for Trial 2, rotating the arena 90° in the opposite direction. Once these two pre-translation test trials were completed successfully, the mouse was trained for two or three more nest box transfers and then tested in the post-translation test.

Post-translation test*: spatial translation of the arena*

The purpose of the this test was to determine the degree to which an experienced mouse can extrapolate spatial knowledge acquired in one explored place to another unexplored nearby place, from which all the same landmarks were visible, but from a different perspective (fig. 1). In brief, do mice have the ability to use viewpoint extrapolation to navigate to a previously approached goal?

To test the mice's directional orientation in an unknown area, the arena was passively translated to a new position. This unfamiliar, new position (Position 2) was located directly north of the original arena location (Position 1) by 1.5 m, i.e.,

the diameter of the arena, (fig. 2a). To test the mice, the arena was shifted from Position 1 to 2 while the mouse and litter were held inside the nest box over the arena. After the arena translation, it was rotated 90° CW or CCW. Then the full nest box was placed in the center of the arena, and the lid removed. the direction each mouse took their first pup (crossing the virtual threshold 15 cm away from the periphery) was recorded, and then the arena was immediately rotated and translated back to Position 1 to avoid having the mouse explore and learn about the new place. The mouse then was allowed to retrieve at least one more litter of pups to the nest box at 0° in Position 1. Finally, the translation/rotation procedure was repeated, but the arena was rotated 90° in the opposite direction in Position 2 for the second trial of this test. The mouse and litter were placed back into the housing cage for the remainder of the day.

Covered or Uncovered Nest box

Hypothetically, the effects of visually perceiving and fully integrating passive translation may or may not affect the mouse's ability to extrapolate spatial knowledge to the new viewpoint. This visual perception could occur during nest box transfers and when the full nest box was held above the arena during arena position changes. Therefore two subgroups within Experiment 1 (pre and post-translation tests) were designated. The *covered* group experienced an obscured view out of the nest box, via an opaque paper covering the nest box door, whenever the nest box was lifted and moved with the mouse and litter inside. The *uncovered* group were handled the same as the covered group, but with no attempts to obscure

the view out of the nest. Of the 30 mice used, 14 mice were in the covered subgroup, and the remaining 16 were in the uncovered subgroup.

Statistical Analyses

Circular statistics were used to analyze the directional data collected for both pre- and post-translation tests, each trial (1 and 2) of both tests, and then within the two subgroups, covered and uncovered. [Only the 21 mice that were tested with the arena rotation in the pre-translation test were statistically analyzed.] For each group of data the mean vector angle and length were calculated with the program, Oriana 3. The angle of the vector (μ) represents the mean angle of the directional choices of the mice. The length of the vector (r) is inversely proportional to the variance of distribution around that mean angle. In other words, as the length of the mean vector approaches 1.0, the less variance in the data (Batschelet, 1981). A Rayleigh's test was then used to test if each mean vector length differs significantly from 0, meaning a random spread of data around the circle (the null hypothesis).

Further analysis was used to compare the pre- and post-translation test results. Because the trained direction and the test for correctly using viewpoint extrapolation were both along the 0–180° axis, any divergence from this axis is an indicator of confusion. Therefore, to test if the horizontal spread away from this axis significantly differed between the first trials of the pre- and post translation test groups, the horizontal components were isolated from the circular data. The cosine of each datum angle gave the horizontal lengths for each mouse in the pre- and post-translation tests. Then the Wilcoxon Mann Whitney rank-sum (WMW) statistic

tested the null hypothesis that there was no difference in horizontal lengths between the two groups.

Behavioral Details

For both the pre- and post-translation tests the pup retrieving behaviors of each mouse was noted and will be reported in the results. Although these behaviors will not be quantitatively analyzed, the presence of a specific behavior can be revealing of a mouse's ability to navigate during the test. For example, a disoriented mouse will drop her pup in an effort to reorient and search for the shelter; whereas, mice that were thoroughly trained to a goal location typically pick up their pups and walk directly toward the goal without hesitation.

Experiment 1: Results

Pre-translation of arena

The retrieval behavior of the mice was to pick up the pup and walk directly toward the periphery without any change of direction before passing the pre-determined virtual threshold for data collection. All of the mice used in this study were successfully trained to retrieve a pup from the center release site to the goal located north at the arena periphery in Position 1 (fig. 3a). The data from the two subgroups, covered and uncovered, were lumped together because the mice were only tested after training was successful, and there was no difference in the amount of training needed. Therefore, visual or the lack of visual access out of the nest box during transfer did not affect these results. The mean vector lengths for both trials significantly differed from random, and indicate very little variance around 0° north (Table 1).

Table 1. Statistics for both trials of each test in Experiment 1, and the covered/uncovered subgroups. Wilcoxon Mann Whitney (WMW) shown when used.

	n	Trial	Mean vector (μ)	Mean vector (r)	Rayleigh's Test p-value	WMW p-value
Pre-translation	21	1	1.47°	0.97	8.65×10^{-9}	
	21	2	-8.76°	0.96	1.19×10^{-8}	
Post-translation	30	1	13.03°	0.52	0.00021	9.5×10^{-5}
	30	2	-4.09°	0.38	0.011	
Post-trans. uncovered	16	1	12.04°	0.67	0.0004	
	16	2	3.38°	0.47	0.027	
Post-trans. covered	14	1	15.21°	0.35	0.19	
	14	2	-17.43°	0.30	0.29	

Post-translation of arena

When placed into the center of the arena in the novel position, the mice's pup retrieval behavior showed confusion about their directional heading. Although some mice walked in a straight path toward the arena periphery, other mice indicated confusion by picking up a pup, heading in one direction, and then coming back to the center and going in a different direction. Also, occasionally a mouse would drop her pup. In that event, the datum angle collected would be the mouse's direction without a pup after she first crossed the virtual threshold, under the assumption she was searching for the nest box in that direction.

The mean vector angles for both trials of this test were toward the trained north direction; the length of the mean vector of the first trial in Exp. 1 decreased significantly compared to the pre-translation test. The variance was greater in Trial 2 than Trial 1 (see mean vector lengths in Table 1); however, for both trials the mean vector length differed significantly from random (Rayleigh's test).

When the data for the post-translation test were separated according to the subgroups, only the mean vector length of the uncovered group was significant (Rayleigh's test). The mean vector angle for the *covered* group was toward the north, but was not significant (Rayleigh's test).

Experiment 1: Discussion

The two main purposes of this experiment were to verify if house mice used extra-arena or intra-arena cues for compass orientation (pre-translation test) and to test if mice used viewpoint extrapolation to navigate a novel shortcut home (post-translation test). The significant mean vector length toward the trained north direction (Fig. 3a) verified previous studies that mice rely on distal visual cues for choosing their directional heading while navigating back home (Alyan and Jander, 1994, 1997). Because of this reliance on the extra-arena cues, there was the potential for the mice to use viewpoint extrapolation because the arena was shifted to a location that shared all of the same visual cues outside of the arena.

In the post-translation test the three hypothetical outcomes were (Fig. 2a): a) the mice would continue north in the previously trained direction; b) the mice would use viewpoint extrapolation to navigate south, back to the known goal; or c) confusion in the unknown place would be shown by a random spread of directional choices in the arena. In short, the mice tended to head north, the same direction as before the arena had been shifted; but also the mice's directional choices away from north significantly increased compared to the pre-translation test. Therefore, there is no evidence that the mice used viewpoint extrapolation, however they maintained their compass orientation and displayed some confusion (Fig. 3a, b).

Possibly, allowing the mice visual access through the nest box door during relocation (uncovered group) affected their directional choices by allowing them to maintain their compass orientation during the transfer. When blocking visual access from the nest box while relocating the arena and the mice (covered group), the mice showed a tendency to walk north, but the mean vector length was insignificant (Table 1). Hence, the opportunity to see while being moved was not used to update the self-location of the mice.

I also cannot rule out the use of vestibular input while moving the nest box with the mice inside. However, if this were the case, I would expect the mice to acknowledge the move to the new position during passive translation and navigate south. Also, when visual cues are present, they tend to override self-motion cues (Alyan and Jander, 1994).

If the mice were unable to self-locate using viewpoint extrapolation in this novel place, then the data were expected to be random, symptomatic of the mice's directional confusion in this unknown location. So the results of the post-translation test in Exp. 1 showing a northern directional trend were surprising—perhaps indicating the mice could not recognize the test position as distinct from the training position because they were too close and shared the same compass cues. Maintaining their compass heading in the novel location is similar to behavior previously described only in ants and honeybees, which is best termed virtual vector orientation (Brun, 1914). Virtual vector orientation was observed when ants were trained to locate a goal in a specific direction and distance from a starting position, but when the ants were relocated to a different start position, they moved

as if they were in the same place by walking the same vector in an attempt to reach the goal.

Experiment 2: Methods

This second experiment was to test whether the mice could learn to visually differentiate between Position 1 and Position 2, relying exclusively on the extra-arena cues. Therefore 24 of the 30 mice from Exp. 1 were trained and tested on a second day, immediately following each mouse's use in Exp. 1. Training began in Position 1 for 30–50 retrievals with the nest box at 0° north. Then the arena was shifted to Position 2, while the nest box was held out of the arena (Fig. 2b). The nest was put in the center of the arena; the lid removed; and the mouse was trained for 30–50 retrievals to a nest box placed at 180° (south) in the arena. An average of 150 retrievals was performed at each position by continually shifting the arena and incrementally decreasing the number of retrievals between position shifts by one nest box switch, which was determined by the litter size for each mouse.

After training, each mouse's directional heading was tested in each arena position. For one position test, the arena was moved to the alternate position of its current location, e.g., from Position 1 to 2, so that the mice must recognize the location change if they were to navigate toward the trained nest location for that arena position (Fig. 2b). The following example of the series of testing between the two positions will better explain the procedure. If training ended in Position 2, then testing immediately followed by shifting the arena to Position 1 and rotating it 90° CW, while the mouse and pups were held out of the arena in the nest box. Then the full nest box was placed in the center of the arena and the lid removed. The mouse

was allowed to retrieve one pup to the periphery (the directional heading noted within 5°) and then the arena was immediately rotated to its original orientation for Position 1, as an empty nest box was placed at 0°. After the mouse retrieved the remaining litter of pups, the full nest box was held above the arena platform while the arena was shifted to Position 2 and rotated 90°CW. Again, the nest was placed in the center and the lid removed. The mouse was allowed to retrieve one pup to the periphery before the arena was rotated back to its original position at Position 2, and an empty nest box placed at 180° for the rest of the litter retrieved. As in Exp. 1, two trials were performed for each test. Therefore, this procedure was repeated twice more rotating the arena 90° CCW in both positions.

Statistical Analyses

For each group of data, circular statistics was used to compute the mean vector angle and length. A Rayleigh's test determined if each mean vector length significantly differed from random.

Experiment 2: Results

Typically the retrieving mice walked a direct path to the periphery; but occasionally the mice changed directions or looped around until passing the pre-determined threshold for data collection. The mean vector angles of both trials show that the mice headed south in Position 2 and north in Position 1 (fig. 4). The mean vector lengths significantly differed from random (Rayleigh's test; Table 2) for both arena positions.

Table 2. Statistical results for Experiment 2 (both trials).

	n	Trial	Mean vector (μ)	Mean vector (r)	Rayleigh's Test p-value
Position 2 to 1	24	1	-6.13°	0.82	6.1×10^{-8}
	24	2	-1.26°	0.83	4.6×10^{-8}
Position 1 to 2	24	1	182.95°	0.91	5.5×10^{-9}
	24	2	180.66°	0.92	3.8×10^{-9}

Experiment 2: Discussion

The purpose of Experiment 2 was to determine if the mice were able to visually distinguish between the two arena positions. And the results clearly showed the mice were capable of this discrimination between the two places after training. Training provided the mice with enough locomotor experience in both places to identify their location and correctly discern the directional heading needed to take them to the goal.

General Discussion

The results from Experiment 2 ruled out the explanation that the northern directional trend in the novel position (post-translation test) was because the mice did not recognize they were in a different location from training. Instead, these results provide evidence that house mice are capable of maintaining compass orientation in a nearby, but previously unexperienced place. To navigate, an animal must be able to self-locate and compute the needed direction toward a goal from the current location. An animal capable of viewpoint extrapolation would to successfully do both in a novel place with familiar visual cues. The results of this study show that while unable to use viewpoint extrapolation, maintaining compass

orientation via virtual vector orientation was not place-dependent. This incomplete extrapolation of spatial information is an important discovery because no previous study has considered the two components of navigation separately.

Previous studies testing for viewpoint extrapolation in rats

I mentioned in the introduction that no study in house mice has explicitly tested for a shortcut mechanism for navigation that is purely map-based. An important stipulation for testing is that the rodent has no prior experience in the novel viewpoint, and furthermore no prior experience along the novel route to a goal. Almost all previous studies focusing on map-based shortcutting abilities in rats have not provided a testing space that is completely unexplored by either not shifting the arena (Morris, 1981) or only shifting it by its radius (Akers et al., 2007, Hamilton et al., 2007, Hamilton et al., 2008) from the training location. An overlap between the training and testing spaces gives the subject the opportunity to explore this space prior to testing, meaning any shortcut seemingly computed is not purely novel.

For example, Morris' (1981) study is persistently cited as evidence of pure map-based navigation because in his water maze task rats navigated from a novel release site to a trained goal. Interpreting this result as evidence that the rats could compute a novel shortcut to the goal is incorrect because he did not shift the arena to a novel space. Therefore, no release site within this space is truly novel to the rats.

Another consideration for testing viewpoint extrapolation is allowing the subjects full visual access to an enriched environment. Many studies attempting to

test rodents have used opaque walls, limiting the subjects' visual access outside the testing arena. Any lack of evidence for viewpoint extrapolation could then be attributed to insufficient visual cues for the subject to make such a complex computation, or insufficient cues for the subject to distinguish between the training and testing locations. This study's experimental design, e.g., transparent arena walls, provided the mice enough visual access to environmental cues to use viewpoint extrapolation, if they were capable. In fact, I provided the blackboard as a salient visual cue to potentially provide distance information because when the arena was moved by its diameter directly north away from the board, its perceived size is then halved. Apparently, the mice did not or could not use this conspicuous cue this way.

Hamilton et al. (2008) acknowledged the importance of full visual access to the surroundings and in an experiment using the water maze task raised the water level and added a transparent wall. Their results indicated the rat had an ability to learn place navigation and extrapolate to a novel location, which seems to contradict our results. However, they only shifted the testing arena by its radius allowing access to half the space previously explored by the rats.

Evidence for map-based shortcuts

In this study, the mice clearly failed to employ map-based shortcuts, despite given optimal opportunity to do so. Such a failure is taken by many as proof for the absence of a fully developed cognitive map—however, the absence of a behavior is not proof it does not exist at all. Furthermore, a truly novel, map-based shortcut has not been demonstrated in any non-human animal, to date. All those who falsely

claimed the contrary collectively failed to rule out alternative novel shortcutting by means of beacon orientation and/or by passive and active path integration. Tolman himself, the creator of the cognitive map concept, did not yet know about path integration, and he additionally ignored the fact, in his much cited shortcut experiment, that a light bulb near the rat's goal could have served as an effective beacon (Tolman et al., 1946a). Therefore one might think home range representation by a so-called cognitive map is not in evidence in any non-human animal.

Is this warranted? The decisive misconception is the notion that there can be only one type of cognitive map, one that exists either full-fledged or not at all. This un-biological attitude naively ignores both the gradual construction of the cognitive map during exploration and the strong inference from universally accepted biological tenets that complex cognitive mapping faculties must gradually advance during ontogeny and phylogeny.

What is the theoretical solution? There is a simple, highly effective extraction from this longstanding conundrum: clearly label respective cognitive maps by their established contents. Thus, the cognitive maps of house mice and other rodents are evidently route-place maps. If more map types are claimed, unambiguous evidence must be provided.

Two components of route-place maps: place and directional knowledge

While no study provided proof of a rodent's ability for map-based shortcutting, there is evidence that rodents navigate via some form of mental route-place map. The evidence for this mental map is seen as rodents successfully

maneuver around in space that has been thoroughly explored by the animal, navigating to goals not seen from the starting point and not via path integration. This route-place map does not suggest that rodents are capable of novel shortcuts, but that acquired spatial information has been organized in memory for later use. This spatial information allows an animal to self-locate using prior knowledge about the distal and proximal cues linked to a specific place, or place recognition, which can then be linked with the known route—the distance and direction—needed to navigate to a goal.

In the mammalian forebrain, place cells and head-direction cells have properties that can be linked to these two behaviorally established route-place map components, specifically place and directional knowledge; that can be recognized as constituents of an animal's navigation behavior. The hippocampal place cells, which exhibit place-dependent properties and are encoded for specific known "place fields" (O'Keefe and Nadel, 1978, Muller, 1996) can be associated with navigating via place recognition. Head-direction cells, found in the entorhinal cortex, are place-independent and have a peak firing range within a specific compass direction of the organism's head (Taube et al., 1990, Taube, 2007), and can be linked to the directional response in navigation behavior (Dudchenko and Taube, 1997), and the place-independent compass control I discovered in mice. Most place cells prefer proximal cues (Siegel et al., 2008) and head-direction cells prefer distal cues (Zugaro et al., 2001), if available.

Successful navigation depends on the place-direction link associated with the two types of information these cells encode. The properties of these two cells types,

one dependent upon location and the other not, suggest that it might be possible to separate the place-direction link. My experimental set up lacked conspicuous proximal cues (intra-arena cues). Therefore, all cues used by the mouse were distal cues (extra-arena), forcing the place cells and head-direction cells to use the same set of cues. Implicit evidence from my experiment would indicate that these place cells are not able to transfer with the visual cues, but that the head-direction cues maintained their relationship to the visual cues in a shifted location.

By distinguishing between the behaviors associated with place knowledge and directional knowledge, my experiment allows me to reevaluate the rodent's ability for viewpoint extrapolation. Complete extrapolation of spatial information necessary for navigation would have been evident by the mice recognizing their current location in the unknown Position 2 during the post-translation test and headed back to the known goal. On the other hand, if no spatial information was maintained in the novel positions, then the mice would search randomly for the goal. However neither option occurred (Exp. 1), thus it seems the compass orientation was maintained with no place knowledge.

The significant northern heading of the mice in Exp. 1 provides evidence that the behavioral components of navigation related to place and directional knowledge can be independently linked to features of the same set of visual distal cues. My results show that in a completely novel place within a known environment, compass orientation is maintained or extrapolated from an area providing the same distal visual cues; but evidence does not support that place recognition can be

extrapolated. If place recognition cannot be extrapolated, then locomotor exploration is required for a rodent to acquire and use place recognition.

The importance of locomotor exploration for rodents

It is important to note that exploration can be performed in two distinct ways: pure visual exploration from one place and locomotor exploration, and both may be combined. In *pure visual exploration*, the mental mapping of the surrounding topography takes place by visual scanning from one or more vantage points. Locomotor exploration occurs as an animal physically moves about an environment, such as when a rodent directly approaches a novel object or moves around in an unknown place. An animal computing a novel shortcut during map-based navigation must be using pure visual exploration to acquire the needed spatial knowledge because any prior locomotor experience means the shortcut is not truly novel. The results from Exp. 1 and 2 support the need for locomotor exploration in an area before successful navigation can occur in rodents.

Studies support the importance of locomotor exploration for rodent navigation. Rats or mice prevented from physically maneuvering through an environment are unable to navigate within that environment even when able to visually perceive it (Sutherland et al., 1987, Alyan, 1994). In the water maze task, rats that could view both halves of the room, but not move in both halves of the pool, were less successful at navigating to the platform (Sutherland et al., 1987). Similarly, a mouse able to view an environment from its nest without moving through the environment or moving through an environment that shares the visual cues where the mouse was tested were unable to navigate home (Alyan, 1994).

Neurophysiological data support the importance of locomotor exploration for rodent navigation. Place cells encode place fields only after rats are given a chance to move about and explore an area, and no place cells are encoded for an area in a box adjacent to the experienced area until after locomotor experience (Wilson and McNaughton, 1993). Thus the neurophysiology of place cells supports the behavioral findings in the current study where the mice, shifted to an unknown area, showed no place recognition, until after training in both positions. Head direction cells are not dependent upon location and show relative stability in firing direction when the animal moves from a known to a novel place (Taube and Burton, 1995), but when rats are passively transported between two positions the cells' firing direction shift an average of 68° (Stackman et al., 2003), which signifies that locomotion is important for rodents. Also, when deprived of optic flow when actively or passively moving to a novel location the shift of firing direction in rat head-direction cells increased slightly (Stackman et al., 2003), which may explain the increase in variance seen between the covered and uncovered groups during Exp. 1.

Primate and rodent spatial cognitive abilities

The controversial existence of map-based navigation is the focus of many studies on different taxa of animals, driven in part, because of the wish to understand human spatial learning and memory. Brain recordings and lesion studies on human and non-human primate brains verify that the hippocampus complex is important for spatial orientation and learning as in rodents (O'Keefe and Nadel, 1978, Hartley et al., 2000, Hampton et al., 2004, Stringer et al., 2005, Zacks

and Michelon, 2005, Jeffery, 2007b, Lavenex and Lavenex, 2009, Nardini et al., 2009), thus comparative analyses among these groups are appropriate for understanding human spatial learning and memory.

Sifting through the many terms used to describe the same concept across disciplines can make comparisons difficult. For example, view-based navigation (Cheung et al., 2008) also called the snapshot theory (Collett and Cartwright, 1983) states that the animal uses a form of image matching to compare 'snapshots,' or stored retinal images, taken when leaving the home base. To navigate home, self-location involves comparing the perceived current retinal input to the stored images while the animal moves until the images visually match (Collett and Cartwright, 1983, Benhamou, 1996, Collett and Collett, 2002, Wang and Spelke, 2002, Cheung et al., 2008, Stürzl et al., 2008). This theory is the same basic idea as viewpoint extrapolation in rodent studies, which is more commonly referred to as instantaneous transfer (Morris, 1981). Though, adding to the multitude of terms, I prefer viewpoint extrapolation; because, for one thing, it is less ambiguous.

Many studies comparing primate and rodent cognitive topographical maps suggest that the two groups can spatially orient similarly (Wang and Spelke, 2002, Lavenex and Lavenex, 2009); but their differences need to be assessed too. One discovery in the primate hippocampus, that has not yet been found in rodents, is the view cell (see review: Rolls, 1999). View cells fire with respect to where a monkey is looking, not the head-direction, eye position or location of the monkey. The same viewing area on a wall elicited view cells to fire regardless of where the monkey was located in the room when viewing it. The properties of these view cells predicts that

enough spatial information can be acquired from pure visual exploration of an area allowing for viewpoint extrapolation.

Similar to rodent studies, no study on primates has tested for viewpoint extrapolation. A test on macaques was performed intending to test their ability for map-based navigation, but the set-up was similar to Morris' (1981) water maze experiment. These monkeys were trained to find food in an open arena in one location and were then tested to locate the food when entering the room from the opposite side, a different viewpoint (Hampton et al., 2004), but with the same training and testing area. Interestingly, both control and monkeys with a hippocampus-lesion were located the reward from the alternate side above chance level, although, both groups decreased performance level. In humans, children at 6 years old are capable of relocating a hidden object when approaching the scene from a different viewpoint, butafter the subjects had the opportunity to fully explore the testing room (Nardini et al., 2009).

One similar concept to viewpoint extrapolation in human studies of spatial orientation is perspective-taking (Zacks and Michelon, 2005, Michelon and Zacks, 2006). This visuospatial transformation describes the ability to predict what someone else can see or what an object or constellation would look like from another viewpoint. Although this prediction does not involve navigation to a goal, it does suggest humans are capable of extrapolating spatial information from a known place to a novel viewpoint with the same distal cues.

Humans may be capable of relocating themselves or objects because they assign environmental reference frames relative to axes perceived in an

environment, allowing us to identify a scene from a novel viewpoint more easily when it is within that intrinsic reference frame (Zacks and Michelon, 2005, Mou et al., 2008). Humans can recognize viewpoints and understand an object array from another viewpoint, but can they use this ability to navigate to a goal?

Sutherland's (1987) experiment with rats demonstrated that only viewing an environment without physically moving through it is insufficient for successful navigation to a hidden platform in the Morris water maze. A replication of his experiment was repeated virtually using humans (Hamilton et al., 2002). Surprisingly, the results for rats and humans were the same. Humans performed better when given the opportunity to virtually explore the entire arena. Replications of this type are important when comparing human abilities to animal models. It is unlikely we could test rodents and probably non-human primates for perspective taking, but it is possible to replicate tests such as my study on viewpoint extrapolation with human subjects. Comparative tests that use virtual simulations, though more convenient, may not be sufficient. That humans may perform better with proprioceptive feedback may explain the results of Hamilton's, et al.. (2002) virtual experiment. It is also important to note that the environment outside of the virtual arena was not enriched with many visual cues. A more natural setting for all animals to navigate within has many environmental cues. These may help humans visually self-locate in an environment.

Whether humans and non-human primates are capable of viewpoint extrapolation is unknown. However, I predict that humans, possibly all primates, would be better at using viewpoint extrapolation than rodents.

Main Conclusion

Mice and other rodents can implement novel shortcuts by beacon orientation and path integration. The demonstration of the proposed third method, viewpoint extrapolation, in this study was negative. Specifically, in the above experiments the mice clearly failed to make novel shortcuts from a previously unexplored place despite visual access to the landmark constellation; in other words, the mice failed to employ viewpoint extrapolation. Various authors had postulated such a third novel shortcut skill, but failed to tell convincingly how such a skill could be experimentally tested.

Also, this study provides evidence that locomotor exploration is a necessary first step for rodents to successfully navigate within a space and future tests should acknowledge this step in the methodology. Finally, another important outcome of this study was behavioral evidence that compass cues are less place-bound than place cues.

Figures

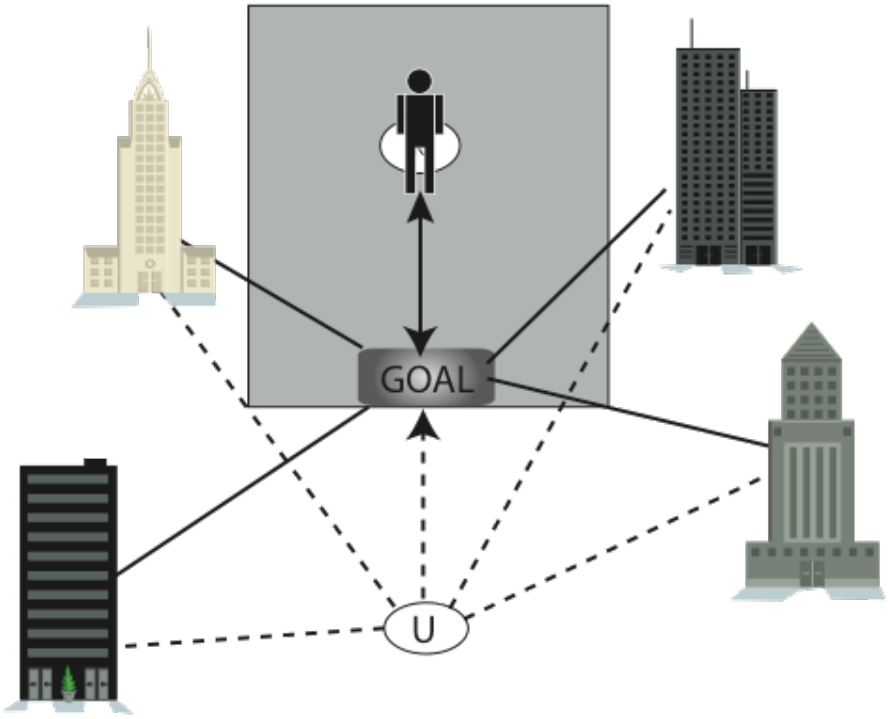

Figure 1. A representation of human viewpoint extrapolation. The shaded region represents an explored area where the person and their goal are localized with respect to distal cues (as indicated by solid lines). After passive displacement to "U," the unknown, or novel, place in the unshaded region, the person infers their location relative to the goal from viewing the same distal cues (dashed lines).

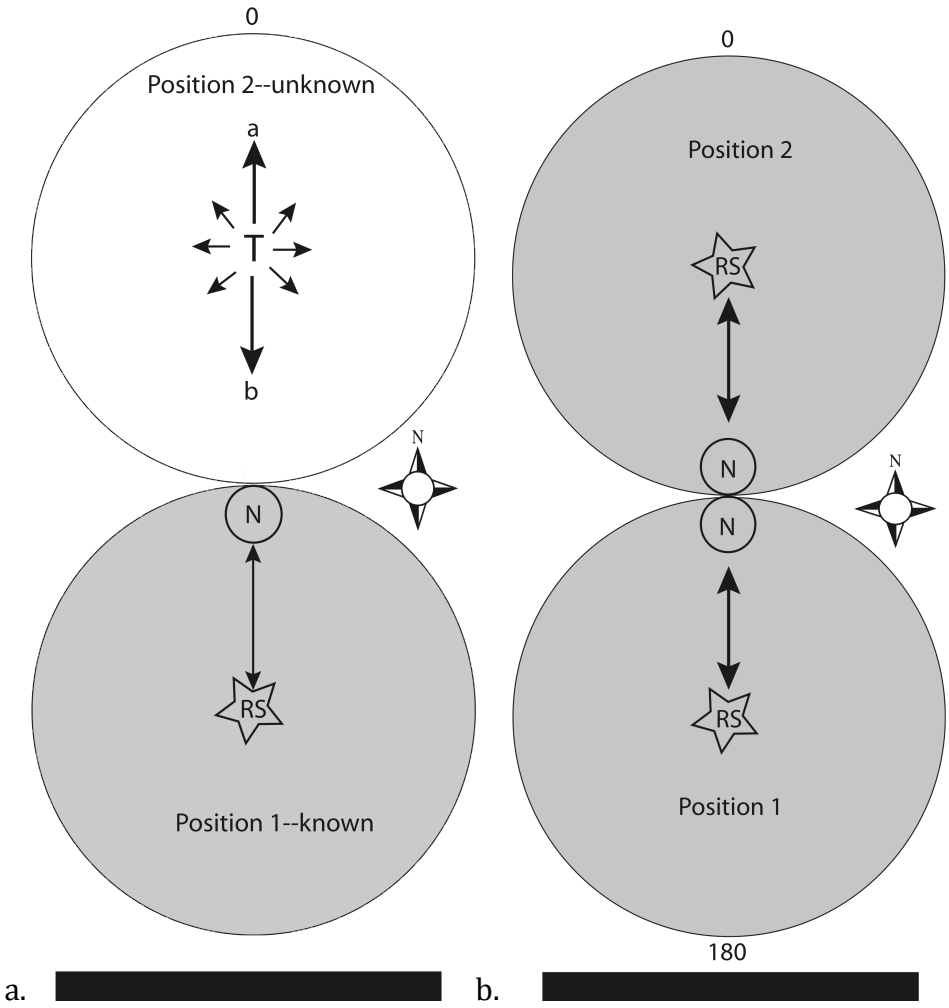

Figure 2. The set-up for the a) the pre-test and Exp. 1, and b) Exp. 2. The shaded circles represent the known areas by the mice; the unshaded circle represents the novel testing position. The solid black rectangle represents the blackboard. The star labeled 'RS' shows the location of the release site where the mouse is initially placed with her litter after a nest box switch during training, and then the mouse walks between 'RS' and the nest box, 'N', to retrieve her pups.

a. Exp. 1: For the pre-test the nest box was removed and the arena rotated either 90° clockwise or counterclockwise. For testing in Exp. 1, the arena was moved to Position 2, and the mouse and litter were placed in the center at 'T', from where she would choose a direction to retrieve one pup. The arrow labeled 'a' is the direction the mouse would walk similar to the training direction, north; the 'b' arrow shows the direction the mouse would walk if using viewpoint extrapolation to navigate back to the nest box. The last option is random choices indicated by the other arrows projecting from 'T'.
b. The Exp. 2 set-up shows the trained direction of the nest box in each arena position. During testing, the mouse and litter were placed in the center (RS), but no nest box was put in the arena.

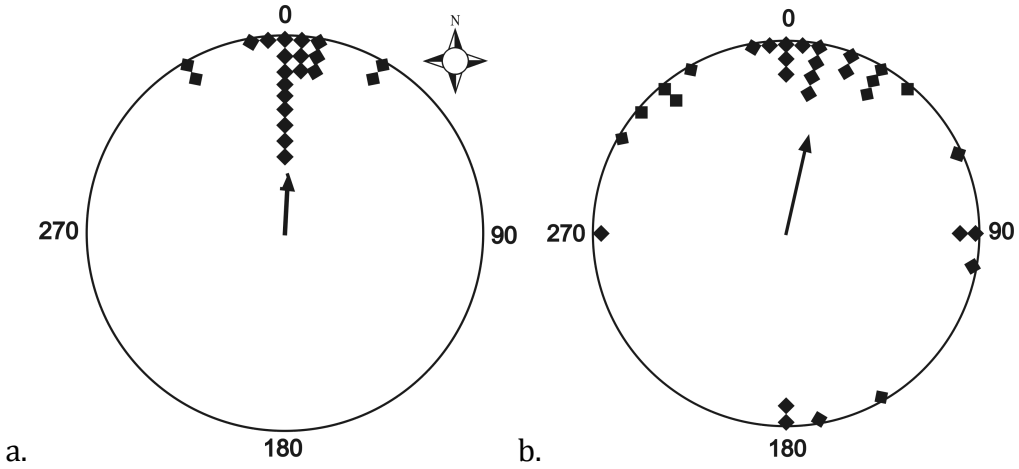

Figure 3. The results from the first trials of the pre-test (a) and Exp. 1 (b). Each diamond represents the directional choice of one mouse. The arrow represents the direction of the mean vector angle (μ), but not the length (r).

a) n = 21, r = 0.97, μ = 1.47°

b) n= 30, r = 0.52 , μ = 13.03°. The WMW p = 0.00009

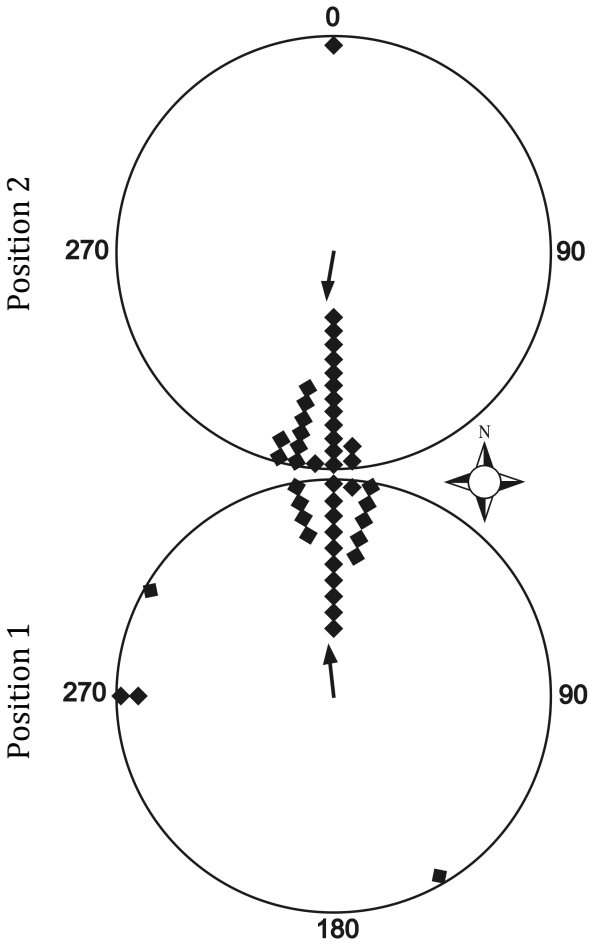

Figure 4. Exp. 2 results. Each diamond represents one mouse's directional choice. The arrow represents the mean vector angle, but not the length. n = 24.
Test in Position 1: r = 0.82, μ = 353.8°. Test in Position 2: r= 0.91, μ = 183.9°

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CHAPTER 5: Can house mice (*Mus musculus*) compute a novel shortcut with two reference viewpoints?

Abstract

The purpose of this study was to continue testing house mice for the ability of novel map-based shortcutting, which tests for the so-called cognitive map. Mice previously failed to use map-based shortcutting when tested for viewpoint extrapolation (Chapter 4); however, the mice were only provided prior experience from one viewpoint before tested at a nearby, unknown location. Perhaps with more opportunity to acquire spatial information mice would be capable of map-based shortcutting. Therefore in this study mice were trained at two locations to move to a goal from different directions, i.e. given two different viewpoints, before being tested in an unfamiliar place directly between these two known places. The mice tested failed to navigate back home via a novel map-based shortcut. In fact, the mice's directional heading was more random than those mice tested for viewpoint extrapolation, indicating more confusion. Therefore, a second experiment was used to both confirm the importance of locomotor exploration for navigating mice and to ensure that the training in Exp. 1, which involved heading to a goal from two different locations, was not the source of confusion in the first experiment. The results from the second experiment showed those mice motivated to explore the entire arena before training started were able to navigate successfully, and immediately, to the goal from a novel test position within the known area.

Introduction

When testing for a rodent's ability for pure mental map-based navigation, it is important to eliminate two alternative mechanisms that would allow the animal to compute a novel shortcut to its goal. Such alternative mechanisms are path integration and beacon orientation (Chapt. 4). Therefore, experiments must be designed to eliminate the opportunity for locomotor exploration in the location where the subject will be tested for novel shortcut ability, because rodents acquire necessary spatial information through locomotor exploration (Sutherland et al., 1987, Alyan, 1994) that makes any shortcut computed not truly novel. A failure to acknowledge this fact led to design flaws in almost all previous studies testing rodents for such mental map-based shortcutting (Tolman et al., 1946a, Morris, 1981, Akers et al., 2007, Hamilton et al., 2007, Hamilton et al., 2008).

To date, one study with house mice (i.e., Chapter 4) correctly designed an experiment that tests the house mouse's ability to compute a novel shortcut back to a goal from a novel place, an ability called viewpoint extrapolation. The results provided no evidence that mice computed a novel shortcut, but suggested that when displaced to a novel place with familiar environmental cues, mice will initially head in a trained compass direction relative to the familiar distal visual cues. This observed virtual vector orientation, or maintaining compass heading, by displaced mice was previously only described in homing ants, which maintain a vector to a goal when moved to a new starting position (Brun, 1914).

The purpose of this study was to provide another opportunity for house mice to compute a novel shortcut to a goal from an unexplored location and to test the

hypothesis that if the mice are unable to self-locate, they instead rely more simply on a previously used heading controlled by a visual compass, i.e., virtual vector orientation. In this study, instead of testing for viewpoint extrapolation, another mechanism of mental map-based shortcutting is tested: *viewpoint interpolation*. Similar to viewpoint extrapolation, the subject would use information about environmental cues seen from other viewpoints to self-locate in a novel place and compute the novel route to a goal. Whereas viewpoint extrapolation uses the information from a single location to apply to a nearby place where the distal cues are shared, viewpoint interpolation requires at least two nearby locations with the novel place between them; so the information acquired from the known locations is *interpolated* for use when viewing shared environmental cues from the novel viewpoint. Possibly, the mice will have more success computing a novel route to the goal when they have two known locations as reference, rather than the one location available to use for viewpoint extrapolation. This study, comprising two experiments, employed similar methods as used to test for viewpoint extrapolation by using the same circular arena to train mice to navigate to a goal.

Methods

Animals

I used 35 experimentally naïve, captive-reared, brown to black female house mice, of which 25 mice were used in Exp. 1 and 10 were used in Exp. 2. They were raised in transparent cages (48 l × 25 w × 15 h cm). Pine shavings and cotton were used as nesting materials, and food and water were given ad lib. The average age of

the mother mice was 8 mo., and the litter sizes ranged from 6–15 pups. All mice were kept under a 12:12 h light:dark cycle and were tested during the light phase.

Basic set-up

Throughout, I used the same circular arena (1.5 m diameter) as described in Chapter 4. In this study, the only change was the absence of the cardboard covering the wooden floor of the arena. Within the arena, north was 0°. The arena was located within a lab room with furnishings enriching the extra-arena visual environment. The room was fully lit with evenly spaced fluorescent ceiling fixtures. The nest boxes—as in Chapter 2 and 4—were a set of two each of a circular, wooden platform and a separate plastic lid that fit together to form a nest box, with an entrance hole cut into one side. Wires were attached to the platform for quick and easy movement of the entire nest box with a mouse and litter inside (see Chapter 2, fig. 1).

General Procedure for both experiments

The same 3-part, intra-arena training procedure (described in Chap. 4) was used to let each mouse navigate from the nest to a goal location and back, using the two interchangeable nest boxes. Once the mouse and litter were inside the nest box at the goal location, this full nest was picked up, and replaced by the empty nest box. The full nest box was placed in one of the designated release sites and the lid removed. Then each mouse retrieved her pups back to the initial nest/goal, one at a time. The goal placement and release sites varied according to the experimental protocol and will be explained in the following sections. Food and water were available in the arena at the periphery, away from the release site and the goal,

during training but were removed for testing. Also throughout training, the observer placed the full nest box in the arena while standing in varied locations around the arena to prevent becoming a reliable visual cue used by the mice.

When testing, the same basic procedure was followed, except no empty nest box was placed in the arena. The quantitative data collected were the directional heading each homing mouse retrieved the first pup, which was measured when the mouse arrived near the arena's periphery by hash marks on the periphery of the arena platform in 5° increments. Only when the mouse crossed a pre-determined threshold of 4 cm away from the peripheral wall in the arena, was the angle recorded. This distance represented where the mouse would encounter the nest box, or be close enough to see it, were it left in the arena.

Visual responsiveness tested

During training, a "vision test" was performed to ensure each mouse could use environmental visual cues to navigate. For this a black, wooden block (8.5 cm × 9 cm × 18.5 cm) was placed upright near the path between the goal and the release site while the mouse was inside the nest box. As novel objects are immediately approached and explored, a mouse that ignored the block was assessed as having poor vision, and that mouse was not used in the experiment. Once the vision test was passed, the block was removed from the arena and training continued.

Viewpoint interpolation experiment

In this experiment, 23 of 25 mice (2 mice were eliminated from the study because 1 failed to pass the vision test and 1 failed to retrieve her pups) were trained to retrieve their pups to a goal location in 2 different arena positions (Position 1 and

2) within the same room. For each mouse, training and testing occurred within one day, averaging 2 hours. No mice were trained together on the same day. During training, the arena was shifted between these 2 positions, while the mouse and litter were held above the arena platform in the nest box. Position 1 was located directly north of Position 2; the centers of the arena positions were 3 m apart (2 arena diameters). The test position was directly between the two training positions; there was no overlap in space between training and testing areas (fig.1).

The nest box locations differed for each training position of the arena. In Position 1, the nest box was placed at 135° (southeast) in the arena and at 45° (northeast) in Position 2. The release site (pup retrieval site) was always in the center of the arena.

For each mouse, the training started with the arena placed in either Position 1 or 2 (alternating from mouse to mouse) and the nest box placed in the respective location relative to the arena placement in the room (fig. 1a,c). First, each mouse was placed in the arena with her litter and the nest box in the respective position, and given 1 hour to explore. Then the general training procedure was followed while alternating between the two arena positions. For example, if training started with the arena in Position 1, then the mouse was trained to retrieve her pups about 50 times to the nest box at 135°. For each mouse, the number of nest box switches (interchanging the full and empty nest boxes) depended on the litter size. Therefore, a mouse with 9 pups would be trained with 6 nest box switches (roughly 50 pup retrievals) and then while holding the full nest box above the arena platform, the arena was shifted to Position 2.

At Position 2, the empty nest box was placed at 45° in the arena periphery, the full nest box placed in the center, and the lid removed. The homing angle the mouse took her first pup in this new position was recorded for later analysis (called *first displacement* data) to check for behavior associated with virtual vector orientation associated with Position 1. The mouse was trained to navigate back and forth between the nest box and the center release site in this new position for about 6 nest box switches. Following the same procedure, the arena was then shifted back to Position 1; and the nest box placed appropriately. This time, the number of nest box switches was decreased to approximately 30 pup retrievals (i.e., 4 nest box switches for the mouse with 9 pups) in each arena position. After these two shifts, the number of nest box switches between arena shifts was decreased by 1 until the arena was shifted between positions with every nest box switch. This procedure was maintained until each mouse demonstrated she could retrieve her entire litter directly to the position specific nest box location with no mistakes for 4 consecutive arena position shifts (2 in each position). Once this occurred, training was deemed complete; and testing followed immediately.

To test the mouse heading in the novel location, the arena was shifted to the center Test Position between Positions 1 and 2 (fig. 1b), while the mice and litter were held above the platform in the nest box. The food and water were removed, and the arena was rotated 180° to control for any intra-arena cues. While the observer stood on the west side of the arena, the full nest box was placed in the center release site; and the lid removed. Then the direction in which the mouse retrieved her first pup was noted (Trial 1). Thereafter, with the mice still in the

arena, the arena was immediately rotated back, shifted to one of the two training positions and an empty nest box placed at the appropriate location for the mouse to retrieve the rest of her litter. After retrieving was completed, the arena was shifted to the other training position, and the mouse retrieved her litter of pups. Then a second trial was performed in the same exact manner as the first.

After an initial look at the results from the first 7 mice tested, their highly scattered headings indicated the mice were very confused at this novel location in the center. This confusion was unexpected because the mice in a previous study (chapter 4) maintained their compass heading, i.e., virtual vector orientation, when placed in a nearby novel location. Because the experimental setup of this study lacked a salient directional cue that the previous study had (the large black board), a salient directional cue was added to the visual background. This salient cue was a directional light source, as there is evidence rodents may use directional light as a compass cue (Fluharty et al., 1976, Etienne et al., 1990, Pass et al., unpublished data). Therefore, for the subsequent mice tested, a 500-watt lamp, 1.5 m tall, was placed at the south end of the room, located 2.4 m away from the south periphery of Position 2 that projected light towards the north over all arena positions (fig. 1). This light was turned on at the start of the exploration hour and left on throughout training and testing.

Experiment testing sufficiency of locomotor experience for shortcut flexibility

The purpose of this experiment was to test if mice could successfully navigate from a novel release site within an area where they had prior opportunity for locomotor exploration, and to determine if training from two release sites to head in

two directions to a goal was the source of confusion in the first experiment. Therefore, the arena remained at the same location for training and testing (Fig. 2). Ten experimentally naïve mice were used that were not used in the previous experiment testing for viewpoint interpolation. One mouse was trained and tested per day, which averaged 1 hour. For all mice the same aforementioned general training occurred at this one location. The mice and litter were given an hour to explore in the arena with the nest box placed at the east periphery, 90°. Then the full nest box (with mouse and litter) was placed at one of two release sites within the arena (RS 1 = north/RS 2= south; fig. 2), and the empty nest box replaced at the eastern goal location. Training alternated between the two release sites after one litter retrieval to the goal. Each mouse's ability to locate immediately the nest box after the initial switch between retrieval sites was noted. Also, the mice were tested for visual ability using a black block as described previously. To test the heading from the novel retrieval site, the arena was rotated 180°; the full nest box placed in the center of the arena; the lid removed; and the direction the mouse took her first pup was noted.

The first 5 mice were trained with a total of at least 100 pup retrievals. During the vision test, the black block was placed in the center of the arena and removed after the mouse approached it. After observing the directional choices of these 5 mice, a new approach was taken. Training decreased to 50 pup retrievals because the mice were demonstrating an immediate ability to locate the nest box from both release sites. After the first two switches between release sites (1 litter each), the black block used for vision testing was placed in the center of the arena.

After the mouse demonstrated visual responsiveness by approaching the black block, it remained in the center of the arena while training continued, because keeping the block in the arena elicited more exploration in the center. The training continued and the black block was removed before the last 2 nest box changes of training, allowing the mice to adjust to its absence, thus not affecting their behavior during testing. The testing procedure was the same as described for the first 5 mice.

Statistics

Mean vector length (r) and angle (μ) were computed for each data set using the statistical program, Oriana 3 (Batschelet, 1981). A Rayleigh's test was then used to test if the mean vector length indicates a significant difference from a hypothetical random spread of directional data around the circle. The significance level was set at the 5% level.

Results

Viewpoint Interpolation Experiment

First displacement

The directional choice of each mouse immediately following the first arena displacement from the known position to the other respective training position not yet experienced (1 to 2 and 2 to 1; Fig. 1) varied widely (fig. 3). The data are separately plotted to show the direction chosen by mice (n = 11) that explored and were trained in Position 1 and then moved to Position 2 (fig. 3a), and the mice (n = 12) that first explored and trained in Position 2 and then moved to Position 1 (fig. 3b). The mice that were trained with or without a directional light source were lumped together because: a) there were very few mice without the light and b) there

seemed to be no difference in the outcome. For the mice moved from Position 1 to 2 (Fig. 3a), the mean vector direction and length were $\mu = 143.24°$, $r = 0.65$, and the Rayleigh's test p-value = 0.007; for the mice moved from Position 2 to 1 (fig. 3b), the mean vector values were $\mu = 252.94°$, $r = 0.31$, and the Rayleigh's test p-value = 0.32.

Descriptively, the pup retrieving behaviors of the mice varied. Some mice walked straight toward the periphery, while others showed confusion, indicated by dropping a pup and/or circling the platform before leaving the center. Also, some mice shuttled repeatedly between the release site and the old goal location before heading to the next box located at the new location in the this new arena position. And then some mice walked back to the old location before finally retrieving their pups into the nest box, which was interesting because they had located the nest box and still walked away, back to where it *had been* before returning to the new location.

Viewpoint interpolation test (Fig. 1b)

The data from Trial 1 are more important because this trial was the true first experience each mouse had in the intermediate test position; Trial 2 data were collected to evaluate consistency of behavior. The results from Trial 1 for all mice (Table 1) show a random spread of directional choices from the test arena position (fig. 4). The last placement of the arena (Position 1 or 2) just prior to testing could have affected the directional heading of the mice in the novel center arena positions. Therefore, the data were separated into two groups by the arena position before testing (fig. 5a,b). The mean vector directions (μ) and lengths (r) were calculated for each group (from Pos. 1 and from Pos. 2; Table 1), and neither vector length was

significant (Table 1, Rayleigh's test). Therefore the arena placement did not significantly affect the mice's heading during the test and was not a factor in the random spread of directional data around the circle for all mice (fig. 4).

Table 1. Mean vector information for Exp. 1. N = number of mice; r = mean vector length; μ = mean vector angle.

		Trial 1			Trial 2		
Group	**n**	**μ**	**r**	**Rayleigh's test (p-value)**	**μ**	**r**	**Rayleigh's test (p-value)**
All mice	23	13.69	0.03	0.99	310.40	0.43	0.01
Light only	16	165.62	0.15	0.70	328.25	0.32	0.30
From Pos. 1	11	284.23	0.38	0.22	301.97	0.58	0.02
From Pos. 2	12	96.03	0.35	0.24	324.96	0.31	0.32

The pup retrieving behaviors of the mice showed considerable directional confusion, i.e., starting in one direction and switching after walking a few steps, or dropping their pup. Occasionally, a mouse would drop a pup and then walk to the periphery. If this occurred, I used the direction the mouse walked past the virtual threshold without a pup as the datum point.

Experiment testing sufficiency of locomotor experience for shortcut flexibility

During training, the first time the release site was shifted to the alternate position from the initial training release site, 9 out of 10 mice immediately departed in the compass direction learned prior to the shift (east, 90°). The only mouse (from the group trained with the black block) that failed was suspected not to explore the arena prior to testing because of a lack of mouse droppings within the arena, which was distinct from most mice left in the arena.

The mice that were trained without the black block in the center of the arena produced a mean vector of r = 0.46, μ = 52.96°, Rayleigh's p-value = 0.37; and data

from those mice trained with the block resulted in a mean vector of r = 0.996, μ = 86.0°, Rayleigh's p-value = 0.001 (fig. 6). The pup retrieving behavior of the mice trained without the black block showed confusion behavior during the test by dropping pups, starting from the center a few times before picking a direction or starting halfway in one direction, then walking back to the center and heading in a different direction. All 5 mice trained with the black block in the center, walked directly from the center to the periphery with no behavior indicating confusion.

Discussion

First displacement of the arena

After the first set of training at one location, the data collected on the directional choice at the respective second location, showed that only some mice responded with virtual vector orientation at the preceding training position. Virtual vector orientation means the mice would maintain their compass heading in the novel location and head toward the previously trained direction. Those mice shifted from Position 1 to 2 (Fig. 3a) evidenced a significant tendency to head in the preceding trained direction, while those mice shifted from Position 2 to 1 (Fig. 3b) showed no significant heading.

Viewpoint interpolation experiment

The two main purposes of this experiment were to test if mice could use viewpoint interpolation to deduce a novel shortcut home and second to investigate the potential tendency for virtual vector orientation in mice, as seen in Chapter 4. Hypothetically, with only extra-arena cues available, the mice could navigate in 3 different ways when the arena was shifted into the unexplored middle position (fig.

1b): a) to use viewpoint interpolation and walk directly east, b) to exhibit virtual vector orientation and walk in either of the trained directions (45° or 135°), or c) choose random directions, indicating an inability to self-locate. No mice walked directly east (fig. 4), meaning the mice did not use viewpoint interpolation to navigate in this testing design; and no matter how the data were separated no mean vector length was significantly different from random (Table 1). Also, the results do not support the tendency for virtual vector orientation.

The random spread of directional data for Exp. 1 could be explained by a lack of sufficient training, but the mice were only tested after showing the ability to successfully switch heading four consecutive times when shifting training positions, always retrieving the entire litter correctly to the nest, hence were well trained and performed well. This methodology proved successful in training mice (Exp. 2 of Chapter 4) to demonstrate that mice obtained enough visual information to navigate in both places. It was therefore assumed that this methodology would be sufficient for the mice to acquire enough spatial information in both environments to use for viewpoint interpolation—if they were capable of it.

Open Questions

The mice's lack of directional choice toward either of the trained compass headings after shifting the arena between Position 1 and Position 2 in Exp. 1 (fig. 4,5) could be a result of the training design. The mice trained and tested in Chapter 4 demonstrated virtual vector orientation and later showed no difficulty distinguishing between the two arena positions; the more complex design of this study may have added too much confusion. First, in this study the mice were

trained by switching between two arena positions and then testing in a third position that was between the other two. Also, the trained goal locations were angled obliquely to the north-south axis in which the arena positions were shifted, which is different from the design of the previous study (Chap. 4, Exp. 1), which shifted the arena along the same axis as the trained goal location. Plus, the mice were trained to orient to two respective goals in two different directions that differed by 90°, rather than 180° as in Chap 4, Exp 2. The more acute angular difference and the third arena position may have made it more difficult for the mice to maintain compass orientation in the novel position. Also, when previously testing for viewpoint extrapolation (Chap. 4), a salient visual cue, the blackboard, was provided; while in this current study, the salient cue was a light source, which mice will use as a direction cue in a dimmed room (Pass et al., unpublished data). Perhaps the directional light was less helpful for compass orientation than the black board and thus reduced the mice's ability to spatially orient.

To determine if mice are inclined to use virtual vector orientation with this set-up, another experiment will be needed with a slightly altered methodology to eliminate the potential confusion of two training positions. That is, some mice would be trained to navigate to a goal in Position 1 (goal at 135°) and their directional choice tested in both the middle testing position and Position 2 (fig. 1), and different mice would be trained in Position 2 (goal at 45°) (fig. 1) and tested in the other two positions. The directional light should stay in the same placement; but if the results again lack a clear trend for virtual vector orientation, then the blackboard should replace the light as the salient direction cue. If these changes still

elicit random directional choices from the mice, then the hypothesis that mice are inclined to respond with virtual vector orientation in a novel place with familiar distal cues can be rejected.

Experiment testing sufficiency of locomotor experience for shortcut flexibility

The design of this second experiment used the same concept of training as in the first, but without shifting the arena position. The mice had the opportunity to explore before training, and the release sites were at opposite sides (north and south) of the arena, at the periphery (Fig. 2). The intent was to determine if locomotor exploration with all places inside the arena was sufficient for mice to navigate to a goal from any release site in the arena, and that the lack of viewpoint interpolation in the first experiment was not because mice require explicit training between a release site and a goal.

However, the five mice trained without the black block in the center inaccurately navigated to the goal from the novel, center release site (Fig. 6). These results can easily be explained by most rodents' tendency to avoid open areas while exploring (Drai et al., 2001, Whishaw et al., 2006, Avni and Eilam, 2008); therefore when the mice were given the opportunity to freely explore prior to training, it is likely they thereby spent little to no time in the center of the arena.

Therefore, 5 more mice were trained with an object in the center of the arena during most of training, encouraging exploration toward this object and movement through the center of the arena, *but* without any training walking along the route between the goal and the center. The mice could immediately retrieve their pups to the goal location (fig. 6). These results and design of this experiment are similar to

the results of Morris' (1981) water maze experiment with rats, from which he incorrectly concluded that rats are capable of computing novel shortcuts through map-based navigation. Thus rodents are capable of navigating between any locations they had previously been connected by locomotor exploration.

First displacement: locomotor experience sufficient

Further support that locomotor exploration is sufficient for compass navigation is shown by the behavior of mice after the first shift to the alternate release site—not yet experienced during training in the second experiment. For both shifted groups, 9 out of 10 mice were immediately able to walk to the nest box located at the east. Although finding the nest box can be accomplished by following the wall of the arena to the goal, the mice had to choose the correct direction to walk along the periphery, which they did without hesitation. The one mouse (from the group trained with the black block) that did not make an immediate correct decision was suspected of very little movement outside of the nest box during the exploration phase of the experiment.

Neurophysiology of compass orientation

Directional choices of the mice are reliant on their ability to maintain their compass orientation when moving among places. Neuronal recordings from freely moving rodents discovered head direction cells found in the hippocampal complex, which have properties that match the behavior of choosing a heading. Usually reliant on distal environmental and vestibular cues, head direction cells encode directional information and have preferred firing directions dependant on a rodent's directional heading, but not on its location (Zugaro et al., 2001, Taube,

2007). Specifically, the firing of these cell types remain fairly stable as a rat moves into a novel place (Taube and Burton, 1995).

Although the mice do not demonstrate the ability for self-location in a novel place, if these head direction cells maintain compass orientation in a novel place, the behavior exhibited in the viewpoint extrapolation set up (Chap 4) could be virtual vector orientation; however, this behavior was not observed for many of the mice in the first experiment of this study. Notably, the firing direction of head direction cells shifted substantially (averaging 68°) when rats were passively transported on a cart into a novel position (Stackman et al., 2003). If proprioceptive feedback is important for these cells types to maintain directional stability when moving into a novel place, then perhaps the design of this current study, which involves passively transporting the mice within the nest box as the arena was shifted, affects any ability to use viewpoint interpolation and possibly the low tendency for virtual vector orientation.

Main Conclusions

In this study, discovering the hypothetical viewpoint interpolation failed; specifically, the mice chose random directions. Hence I could not find evidence for a pure mental map-based shortcut computation in house mice with this experimental design.

However, the second experiment clearly demonstrated the importance of locomotor exploration for navigation in house mice. That is, mice can compute a vector home from anywhere previously explored via locomotion; thus, emphasizing

the need to take into account whether the mouse had the opportunity for locomotor exploration when designing experiments to test for map-based shortcutting.

Figures

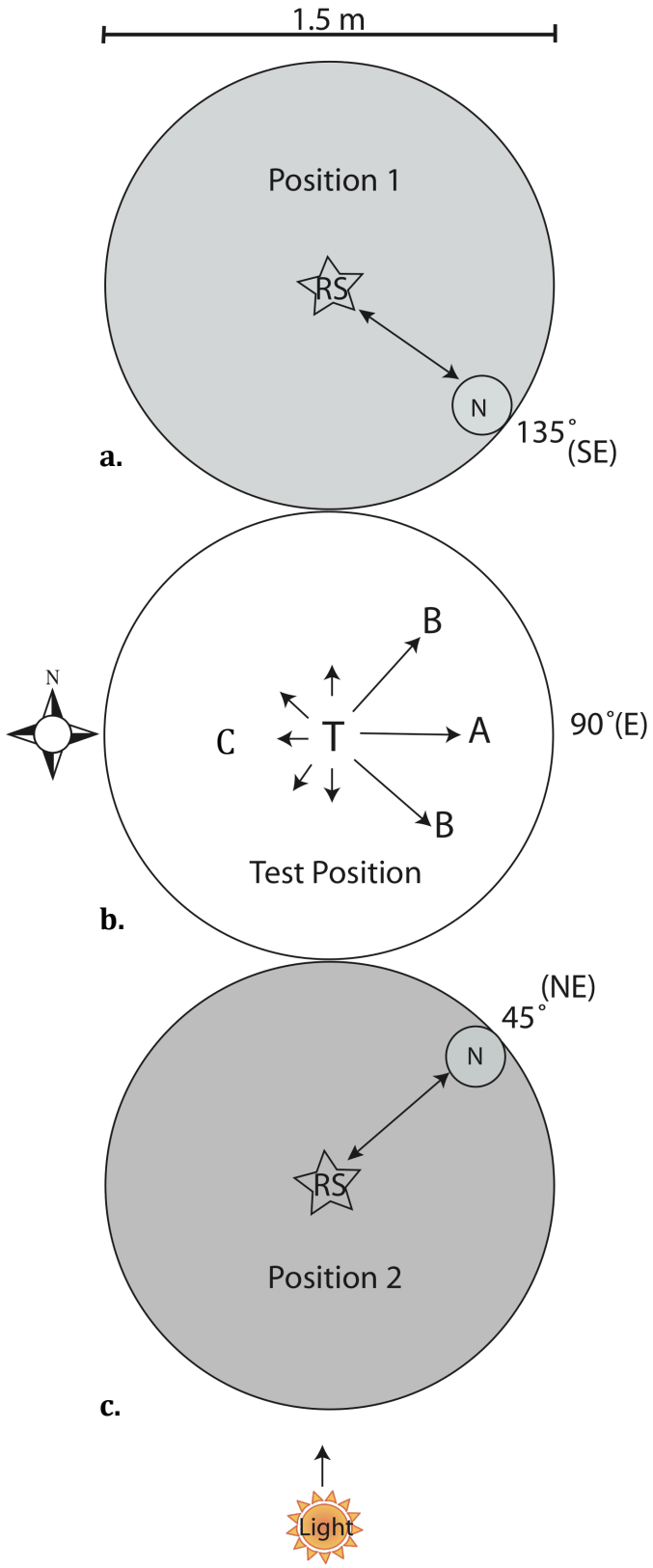


Figure 1. Exp. 1 layout.
The shaded circles represent the two training positions (a,c), with the test position located between them (b). The circle labeled 'N' represents the nest box, the goal, location in each training position. The star labeled 'RS' is the release site where the mouse and litter were placed for the mouse to retrieve her pups to the nest. In the test position, the 'T' is where the mouse and litter are placed for the test. The arrows surrounding 'T' show the possible choices the mouse can walk: A) if the mouse uses viewpoint interpolation, B) one of the two trained directions, or C) the shorter arrows represent all other directions that are possibly spread randomly around the arena. The light source, when used, is aimed at the set-up from the south.

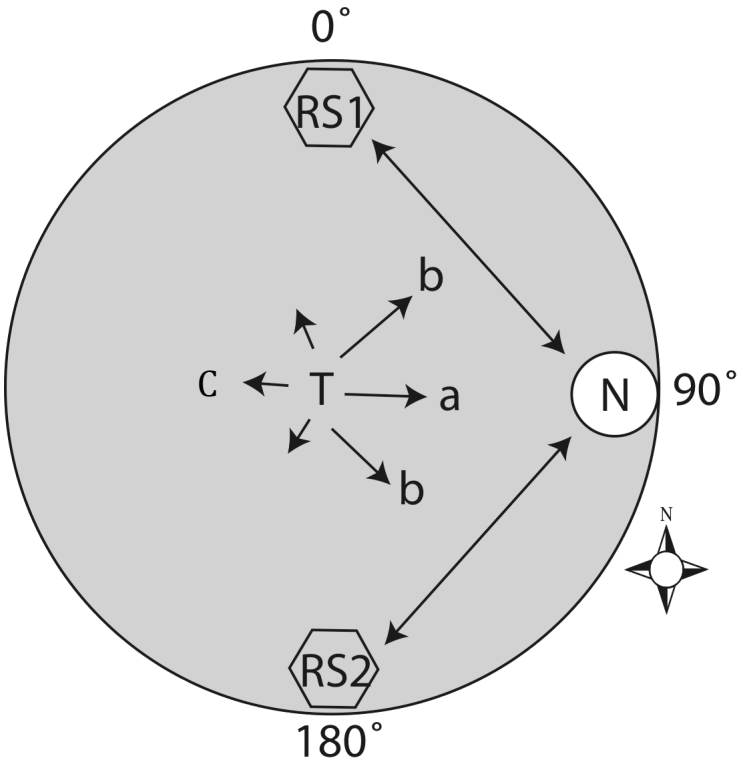


Figure 2. Exp. 2 set-up.
The stationary arena is shown shaded to represent the area experienced through training. The mouse was trained to retrieve her pups from one of 2 release sites (RS) along the periphery at 0° and 180° to the nest box, labeled 'N,' located at 90°. The mouse and litter were placed in the center, 'T', to test the direction she took her pup. The arrows represent possible outcomes: a) toward the goal, b) the trained directions from the release sites, or c) the shorter arrows showing any random direction. During the test, the nest box was not located in the arena.

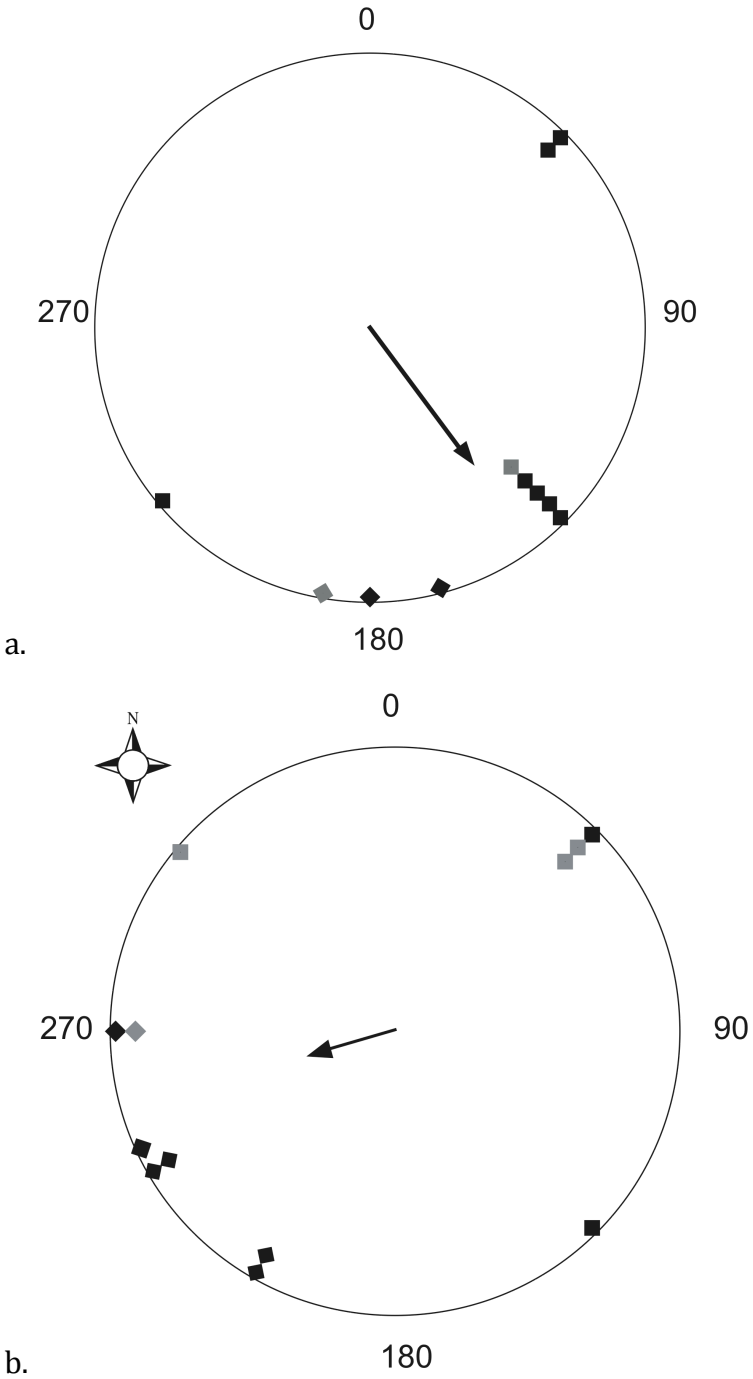


Figure 3. Heading directions of mice after first arena displacement.
The direction the first pup was retrieved for each mouse after the arena was first shifted from its initial position. The diamonds represent each mouse's directional choice; the gray diamonds represent the data for mice before the directional light cue was added to the set-up. The arrow represents the direction of the mean vector angle. **a)** The direction of each mouse (n=11) when shifted to Position 1 from Position 2 and **b)** shows each mouse's directional choice (n=12) when the arena was shifted to Position 2 from Position 1.

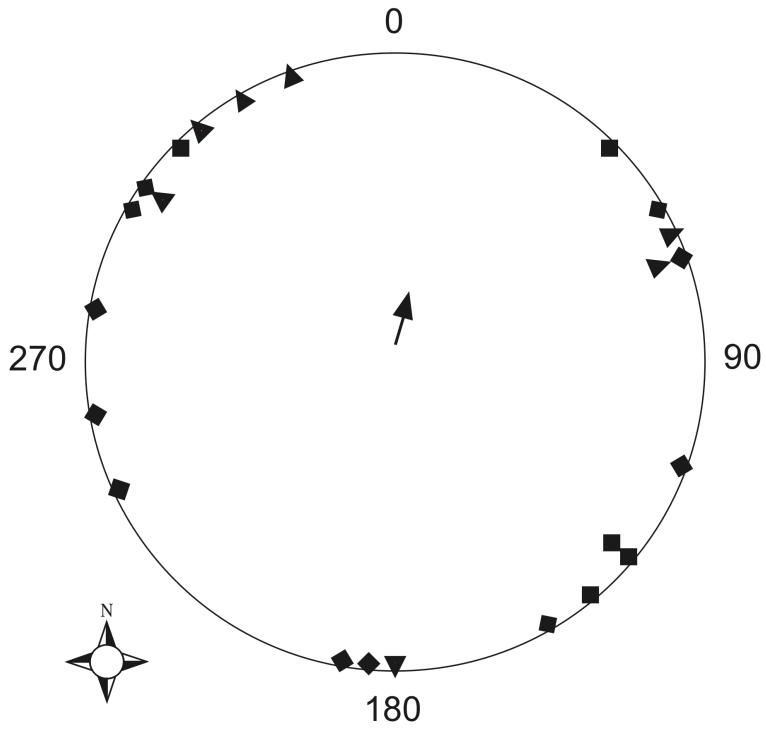
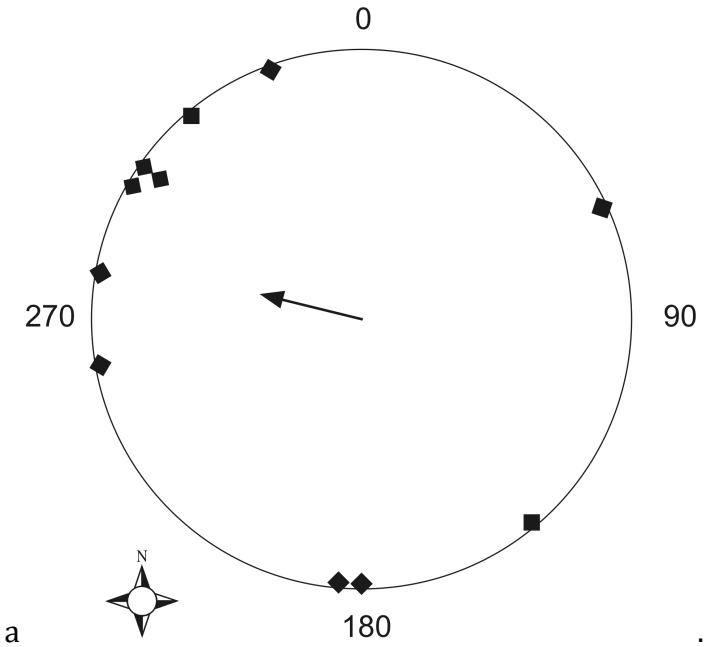


Figure 4. Heading directions of all mice for Trial 1 of Exp. 1.
The diamonds represent those mice with the directional light cue and the triangles represent those mice without the added light cue. The arrow represents the mean vector angle direction of all mice (n = 23, with and without light). Mean vector: μ = 13.69°, r = 0.03



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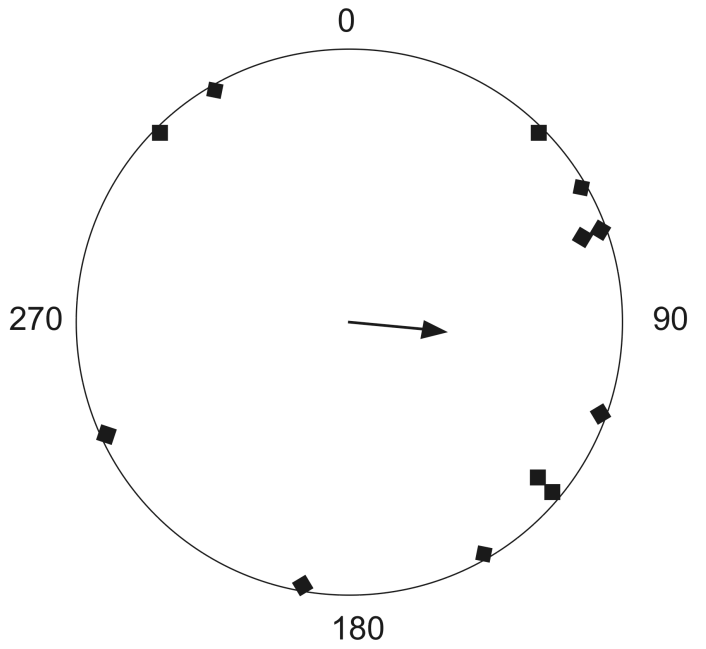

b.

Figure 5.
The results from Exp. 1 separating the data depending on which position the arena was located before shifting into the test position for Test 1. **a)** The mice (n = 11) that were last trained in position 1 before testing. Mean vector μ = 284.23°, r = 0.38. **b)** the mice (n = 12) that ended training in position 2 before testing, mean vector μ = 96.03, r = 0.35. The arrows represent the mean vector angle for the separated data groups.

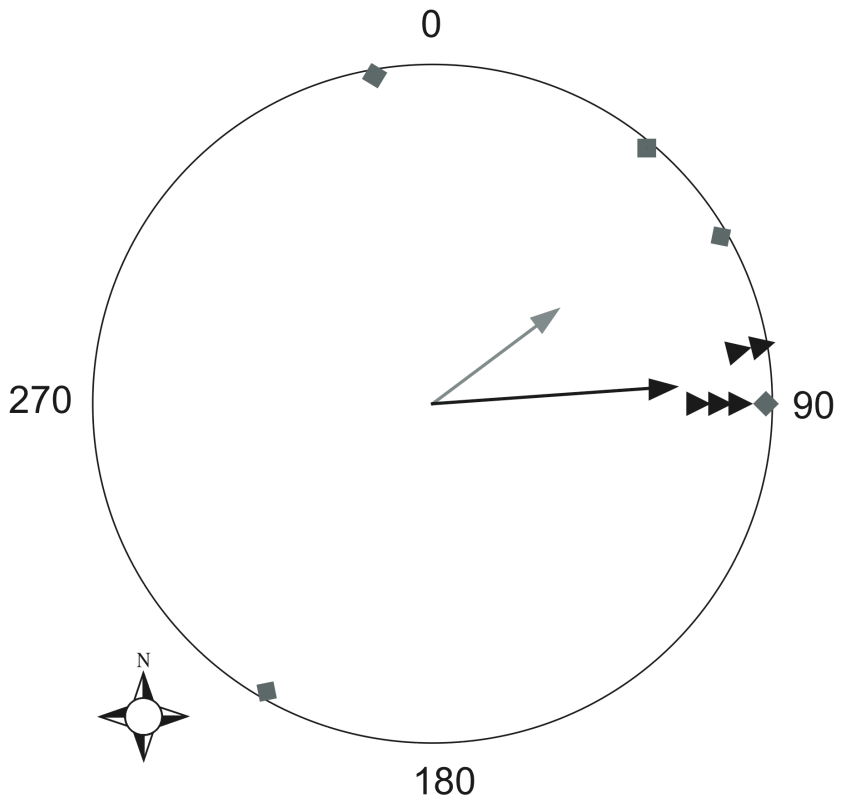

Figure 6. The results from Exp. 2 of both groups of mice tested with or without the black block present during training. The gray squares show the data for mice (n = 5) trained without the black block left in the arena and the gray arrow represents the mean vector angle direction. The black triangles show the data for the mice (n = 5) trained with the black block in the center of the arena, the black arrow shows the mean vector angle.

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GENERAL CONCLUSION

This chapter discusses my dissertation research within two main sections: rodent home range orientation and the broader impact. Within the section discussing how my research added to the scientific knowledge on rodent home range orientation, I outline the primary conclusions, i.e., the most important and novel discoveries of my research, and then the secondary conclusions, which are important but lesser discoveries. Following this outline, I briefly discuss the details of each of the chapters' results and described the importance of these discoveries to home range orientation within three main areas: locomotor exploration, use of learned compass cues for navigation, and testing for a "cognitive map." Finally I describ three areas in which my dissertation research could contribute to a broader level of scientific knowledge.

I. HOUSE MOUSE HOME RANGE ORIENTATION

Maintaining and knowing about a home range, an area of daily activity, is a beneficial necessity to the owner of that space (Stamps, 1995). One major requirement of this familiar space is knowledge about the locations of resources within this range and how to most efficiently navigate among them. These resources include, shelter, escape routes, food, and water. Further, especially for a prey species such as the house mouse, it is also important to know escape routes to shelter to decrease travel distances and predation rates relative to moving around in an unfamiliar space (Snyder et al., 1976, Jacquot and Solomon, 1997). So how is it possible for the house mouse to learn and later use spatial knowledge for successful navigation, i.e., home range orientation?

The work in this dissertation studied previously unresolved behavioral aspects of home range orientation in the house mouse. My more specific focuses were on the learning process (Chapter 1), and the use of learned spatial information for compass orientation (Chapters 2 and 3), and then I proposed and tested a hypothesis based on the highly debated concept of a "cognitive map" (Chapters 4 and 5). The following is a list of the primary novel conclusions that can be made from this research, followed by a list of secondary conclusions.

Primary conclusions:

1) Locomotor exploration has a systematic component.
2) Mice are capable of flexible cue use, which negates the popular snapshot theory.
3) Mice rely on different cues for the outward path and the homing path of a familiar complex roundtrip.
4) Two testable hypotheses designed for the theory of "cognitive maps" are viewpoint extrapolation and viewpoint interpolation.
5) No evidence was found that mice could compute a novel shortcut as defined in the above theories.

Secondary conclusions:

1) Mice will avoid exploring a place previously explored two visits prior.
2) In mice, locomotor exploration is a necessary first step for acquiring spatial information.
3) Salient directional light cue may not be as important as other compass cues for navigating mice.

4) Confirmation that mice prefer to use distal allothetic cues to home under the lighted conditions.
5) It is possible to observe a separation of behaviors associated with directional knowledge and place knowledge—one is place-independent and the other is place-dependent just like the corresponding cell types.

A) Learning through locomotor exploration

In Chapter 1, I separated some systematic from random components of locomotor exploration. Chapters 4 and 5 provide strong evidence that locomotor exploration is necessary for learning in house mice rather than the alternative, i.e., pure visual exploration.

Most past studies of rodent exploration looked at how the arrangement of objects in a novel environment affected exploration (Poucet et al., 1986, Thinus-Blanc et al., 1992, Skov-Rackette and Shettleworth, 2005, Yaski and Eilam, 2007, 2008), or characterized various elementary behaviors, such as, forward movement with frequent stopping and scanning of the environment (Drai et al., 2001, Clark et al., 2006). Very few studies looked for the spatiotemporal pattern of exploration behavior via locomotion to better understand how this learning process helps acquire and organize spatial information for later use. The spatiotemporal pattern is typically described as random. How can a random behavior be useful for acquiring spatial information to be organized into some form of mental map for later use? For example, when you explore a new city, you may not have a specific goal in mind but if you wander around randomly you may not find your way back to

your car. So while locomotor exploration is often described as random, it must have a systematic component.

Past studies that focused on the spatiotemporal behavior of rodent locomotor exploration have done so in an open arena with no home base provided, and the primary result of these studies shows the first activity of these rodents in lighted conditions was to establish some area as a home base (Drai et al., 2001, Zadicario et al., 2005, Avni et al., 2006, Clark et al., 2006, Nemati and Wishaw, 2007). Therefore, Chapter 1 focused on observing any spatiotemporal pattern in a novel arena when a home base was provided, and to simplify the initial observations the exploration activity was limited to a radial arm maze with the home base in the center. With this experimental design, the results revealed that mice explored each arm over time with both random and systematic components. The systematic component was that mice prefer to avoid walking down the same arm of the maze that was visited one or two prior visits, but after that any arm chosen is indistinguishable from random choice.

The systematic component was an important discovery to a previously described random behavior, however, it is also important not to overlook the random component. Randomness is often dismissed in scientific results, but it does have value to a biological system. In this case, the random component to behavior is important to avoid being predictable to predators, especially at a time when an area is unknown or not well known. Future studies can begin to look for other systematic patterns to the movement of mice (or other rodents) in different mazes, an open arena, and eventually a more natural and complex setting.

B) Using learned spatial cues for compass orientation during navigation

Once spatial knowledge has been acquired and the house mouse is capable of navigating to a goal, results from Chapter 2 confirm that mice prefer to use allothetic distal cues (or extra-maze environmental cues) for choosing a heading home, but as newly discovered, if these cues become unreliable the mice can learn to ignore them. Such flexible cue use is important because it shows the ability to continually learn which spatial information maintains relevance. Also, this flexible cue use provides evidence to reject a persistent theory among cognitive scientists: the inflexible snapshot theory.

Snapshot theory states that the animal takes a 'snapshot' of its home, as it leaves; then to return home compares its current perceived retinal input with the stored image or snapshot, and so moves until the two images match (Collett and Cartwright, 1983). This theory persists despite no study ever providing evidence to support it (Collett and Collett, 2002, Wang and Spelke, 2002, Cheung et al., 2008, Stürzl et al., 2008), but the results from Chapter 2 showing the ability of mice to ignore the distal allothetic cues, which would be the entire background of the "snapshot," provide evidence against the theory. The secondary conclusion from Chapter 2 was to confirm, as mentioned above, that mice prefer to home using distal allothetic cues rather than local allothetic cues, which is inherent in the design of experiments in Chapters 3, 4, and 5.

Chapter 3 was the first study to explore the idea that the two parts of a round trip, outward and homeward, rely on a different set of available cues. Also, this chapter delved into an ignored sub-mechansism of navigating along a familiar route

to the animal, which is feedback control. Part of the benefit to an animal of living in a home range is the knowledge about the route to take to reach a goal, which can be pre-formed mentally before embarking. Therefore, a key component to moving along this pre-planned route is confirmation via feedback control that the path is negotiated correctly.

Thus the purpose of Chapter 3 was to determine how different compass cues influence the feedback control while mice negotiate an intersection in a plus-maze along a learned route. The results showed that the preference for distal allothetic cues confirmed in Chapter 2 also influenced feedback control along the homing route, but not during the outward trip. Alternatively, on the outward path, mice will ignore any cue changes that occur while at home and leave the nest on a pre-formed route and only rely on vestibular input for feedback control while en route to the goal. Future studies should define which part of the round trip is the focus of the test for cue use; because so few studies actually look at the outward path, studying the stages involved with the learning process to get such a pre-formed route and which cues are used would be important.

C) Testing for a "cognitive map" via novel, map-based shortcutting

Chapters 4 and 5 test a more theoretical explanation to home range orientation, which is the proposed "cognitive map". From the introduction of the cognitive map theory (Tolman, 1948), which then was vaguely defined, conceptual chaos has ensued that has lasted to this day about whether non-human animals have one or not (O'Keefe and Nadel, 1978, Morris, 1981, Poucet, 1993, Benhamou, 1996, Eichenbaum et al., 1999). A major purpose of Chapter 4 was to clarify the

popular hallmark of a cognitive map, the ability for an animal to compute a truly novel shortcut to a known goal, excluding all other available shortcutting methods (e.g., path integration).

Given this map-use criterion, Chapters 4 and 5 defined two related testable hypotheses for such map-based shortcutting called viewpoint extrapolation and viewpoint interpolation. A key component of both theories was that an animal must have the ability to acquire spatial knowledge from pure three-dimensional visual exploration; i.e., any locomotor exploration within the space tested means the shortcut is not truly map-based. Relying on the preference for distal allothetic cues for compass orientation, both experiments were designed to discover if mice use this ability. Neither experiment provided evidence that mice are capable of such pure map-based shortcutting, despite the fact that the results from Chapter 4 demonstrated that if distal allothetic cues are visually available a house mice in a novel environment could maintain its directional heading. This result showed that of the two important components of correct navigation, place-knowledge and directional knowledge, directional knowledge is less place-bound while place knowledge is strongly place-dependent.

In this context, Chapters 4 and 5 provide evidence that locomotor exploration is exclusively necessary for rodents to acquire the spatial knowledge to successfully navigate within a home range. Plus, when comparing rodent spatial orientation with other mammals, future studies should focus on whether pure visual exploration is sufficient for navigation in a novel area. If so, then that animal may

have a true cognitive map as can be said for humans, who can, for instance, sketch maps without moving based on a three-dimensional vision.

II. THE BROADER IMPACT

A) Adding to neuroethological studies of rodent spatial orientation

Studies on rodent spatial orientation have begun to link their navigational abilities to their neurophysiological attributes, i.e., place and head-direction cells (Quirk et al., 1990, Dudchenko and Taube, 1997, Hartley et al., 2000, Jeffery, 2007a, Taube, 2007). Also, there are some other types of cells that have been discovered in the rodent brain, such as grid cells (Hafting et al., 2005) and border cells (Solstad et al., 2008), which have yet to be linked to the behavioral control of navigation. The purpose and function of grid cells is still unclear, although their firing patterns indicate they probably play some role in navigation (Sargolini et al., 2006, Moser et al., 2008). And new types of cells may be discovered when new firing patterns can be linked with behaviors found in exploration. The more behaviors that can be linked to the firing patterns of distinct cell types, the better neuro-ethological models can be built in order to explain the deep mechanisms of topographic orientation.

B) Behavior ecology implications

Moving in the other direction from neurobiology, this research can be extrapolated to studying how wild animals navigate through their natural home ranges. Lab experiments made numerous discoveries about spatial memory and spatial orientation in animals (especially rodents and primates) that would be impossible to isolate from studies only done in the field. Thus, studies using lab

experiments, such as the work in this dissertation, can be considered in light of the more complex natural world.

Most research on home range behavior tends to map out the area of a home range of an animal as it moved through space and time or determine seasonal movements. However, recently a few studies focused on the routes taken by different species of non-human primates as they move through their home range. These primates have the ability to successfully move in a directed path toward a resource not seen from the starting point (Valero and Byrne, 2007) and the ability to remember when and where a resource has produced food, rather than simply moving along a path and finding the closest food (Cunningham and Janson, 2007).

Also, when avoiding a conflict by detouring around another group, rather than showing "cognitive mapping" ability using a novel shortcut, monkeys tend to rely on distinct topographic features (distal allothetic cues) to return to a well used route from a detour (Noser and Byrne, 2007), and to follow repeatedly used routes within the home range (Di Fiore and Suarez, 2007). In other words, the primates seem to rely on remembered routes and intersecting nodes that allow a route-based map connecting two distant locations that does not necessarily involve the shortest distance (Noser and Byrne, 2007, Garber and Dolins, 2010). More studies of these types on all mammal species that move through a home range are important for understanding how they are capable of learning and using their space.

C) Adding to our understanding of human cognition and pathologies

According to the Alzheimer's Association, 5.3 million people in the United States currently are living with Alzheimer's, which will increase drastically as

millions of baby boomers reach their senior years. Finding a treatment for or a preventive method of this disease and other memory disorders is contingent on understanding the neurological causes.

Episodic memory is the term used to describe the ability to remember specific personal experiences or events that occurred at certain places and time (Tulving, 1983). Episodic memory that is affected by people suffering from Alzheimer's and other forms of dementia, as well as some forms of amnesia. Through hippocampal damage in humans and other animal models, scientists now know that the hippocampus plays an important role in episodic memory, in particular its role in spatial learning and memory (O'Keefe and Nadel, 1978, Muller, 1996, Wood et al., 2000, Jeffery, 2007a, Lee et al., 2008, Moser et al., 2008, Lavenex and Lavenex, 2009), which is associated with home range navigation in rodents.

When trying to understand the function of an area in the brain, the behavior associated with that function must be fully understood so that any behavioral deviations can be correlated with affected regions in the brain. Lee, et al.. (2008) made an important point that most studies on the hippocampus consider it a unitary structure, manipulating the whole structure, e.g., through lesions; but it would be more beneficial to identify the specific functions of individual subfields within the hippocampus. Rodents, specifically rats and house mice, are the representative group used to initially study mammalian brain functions. Once the navigational behavior of rodents is fully understood, the learning of spatial knowledge and the performance from the memory of spatial knowledge can be linked with the neurophysiology of their brain. From that point, the specific subfields of the

hippocampus can be associated with the specific behaviors of learning and memory. This greater understanding could lead to a solution to memory disorders and diseases.

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